

A Multifactorial Genetic Approach to Improving Welfare in the Racing Greyhound

Thesis submitted in accordance with the requirements
of the University of Liverpool for the degree of
Doctor in Philosophy

By

Ruth Jessica Dockerty

January 2016

Declaration

I declare that this thesis was written by myself, the work in this thesis is my own, all material cited has been acknowledged and this work has not previously been submitted for a degree at another university.

Ruth Jessica Dockerty

Acknowledgements

I would like to thank my supervisors, Dr Iain Young and Dr Melissa Royal, for their help and support throughout my PhD. Thank you to the Biotechnology and Biological Sciences Research Council (BBSRC), Biosciences Knowledge Transfer Network (KTN) and the Greyhound Board of Great Britain (GBGB) for funding the project. Thanks also to the British Society of Animal Science (BSAS), Retired Greyhound Trust (RGT) and Higher Education Innovation Fund (HEIF) for providing additional funding for the work.

I would like to thank the Institute of Evolutionary Biology at the University of Edinburgh for allowing me to attend a semester of their Quantitative Genetics and Genome Analysis MSc programme. I am very grateful to Professor Erling Strandberg at the Swedish University of Agricultural Sciences (SLU) for providing valuable training and guidance with genetic analysis of the Greyhound performance and pedigree data.

Thank you to the Centre for Integrated Genomic Medical Research (CIGMR) at the University of Manchester for allowing me to use their labs and equipment, and to Dr Andrea Short for her advice on sample collection and DNA extraction in the Genome-Wide Association Study. I am very grateful to Dr Jonathan Massey for training in DNA extraction techniques, for genotyping the final DNA samples and for all his help and advice. Many thanks also to Dr Robert Coleman at the University of Chester for measuring cortisol concentration in the Greyhound saliva samples.

Many thanks to the Greyhound Board of Great Britain for inviting me to attend and later join their Veterinary Sub-Committee, and for inviting me to present my work at their annual conferences. I am grateful for their continuous support of the project and enthusiasm for application of the work to the British Greyhound racing industry.

I would like to thank all the Greyhound owners and trainers that agreed to take part in the study, and gave up their time to enable collection of samples from their dogs. Thank you to the group of University of Liverpool veterinary science students that assisted with saliva sample collection for cortisol analysis, and to the race track management for supporting the study. Thanks also to Carol Hercock and Diana Hodson for help with collection of tissue samples.

Thank you to my friends for their support and encouragement throughout my PhD and for always being there for me - especially Phil. Finally, huge thanks go to my family. In particular, thank you to my mum and dad, Val and Garry Dockerty, and my sister Hannah for everything - I am dedicating this thesis to them.

Table of Contents

Declaration	i
Acknowledgements	ii
Table of Contents	iii
List of Tables	xii
List of Figures.....	xvi
List of Abbreviations.....	xxiv
Abstract	xxvii
Chapter One.	1
1. Introduction and Literature Review	2
1.1. Greyhound Breed Characteristics.....	2
1.2. History of the Greyhound.....	5
1.3. History of Greyhound Racing in the UK.....	6
1.4. The Greyhound Racing Industry of the UK.....	8
1.4.1. Licensed Greyhound Racing in the UK	8
1.4.1.1. Formation, Structure and Financing of the Licensed Greyhound Industry in the UK.....	8
1.4.1.2. Greyhound Registration	10
1.4.1.3. Greyhound Schooling	11
1.4.1.4. Greyhound Training.....	11
1.4.1.5. Greyhound Racing	12
1.4.2. Independent Greyhound Racing in the UK	14
1.5. Greyhound Racing Outside the UK.....	15
1.6 Whippet Racing	16
1.7. Racing Injuries of the Greyhound.....	16
1.7.1. Muscular Injuries of the Racing Greyhound	22
1.7.2. Tendon Injuries of the Racing Greyhound	24
1.7.3. Ligament Injuries of the Racing Greyhound.....	25
1.7.4. Fractures and Luxations of the Racing Greyhound	26
1.7.4.1. Fractures and Luxations of the Carpus (Wrist).....	30
1.7.4.2. Fractures and Luxations of the Tarsus (Hock)	31
1.7.4.3. Fractures and Luxations of the Metacarpus and Metatarsus	37

1.7.4.4. Fractures and Luxations of the Phalangeal Bones	40
1.7.5. Miscellaneous Racing Injuries of the Greyhound	41
1.7.6. Genetic and Environmental Factors in the Pathogenesis of Stress Fractures	41
1.7.6.1. Genetic Factors in the Pathogenesis of Stress Fractures in Humans.....	42
1.8. Recording and Reporting of Greyhound Injuries	44
1.9. Welfare of Racing Greyhounds in the UK.....	46
1.9.1. Definition of Animal Welfare	46
1.9.2. History of Welfare Concerns in the British Greyhound Racing Industry	48
1.9.3. Welfare Improvements in the British Greyhound Racing Industry.....	53
1.10. Selection and Animal Breeding	57
1.10.1. Genetics of Quantitative Traits	58
1.10.2. Phenotypic Selection.....	60
1.10.3. Estimated Breeding Value (EBV)	61
1.10.4. Heritability (h^2)	64
1.10.5. Genetic Evaluations and Application of EBVs to Livestock Breeding Programmes	69
1.10.6. Genetic Evaluations and Application of EBVs to Equine Breeding Programmes.....	70
1.10.6.1. Thoroughbred Racehorses.....	71
1.10.6.2. Sports Horses	72
1.10.7. Canine Breed Improvement Programmes	73
1.10.7.1. Domestication of the Dog	73
1.10.7.2. Pedigree Dog Breeding.....	75
1.10.7.3. Genetic Evaluations and Application of EBVs to Canine Breeding Programmes	77
1.10.8. Canine Genomics.....	81
1.10.8.1. Sequencing of the Canine Genome.....	81
1.10.9. Approaches to the Genomic Study of Complex Diseases	83
1.10.9.1. Candidate Gene Association Studies	83
1.10.9.2. Genome-Wide Association Studies (GWAS)	84
1.11. Greyhound Breeding	85
1.11.1. The Greyhound Breeding Industry	85
1.11.2. Genetic Analysis of Racing Performance in the Greyhound	89
1.12. Measures of Stress in Canine Athletes.....	92
1.12.1. Physiological Effects of Intense Exercise in the Racing Greyhound.....	92
1.12.2. Cortisol as a Measure of Stress	93
1.12.3. Additional Measures of Stress.....	96

1.13	Summary.....	97
1.14	Aims of the Thesis.....	100
Chapter Two.		101
2.	General Materials and Methods.....	102
2.1.	British Racing Greyhound Pedigree and Performance Database.....	102
2.1.1.	Data Collection and Creation of a Pedigree and Performance Database for British Racing Greyhounds	102
2.1.1.1.	Dog Data Table	110
2.1.1.2.	Pedigree Data Table	112
2.1.1.3.	Performance Data Table.....	112
2.1.1.4.	Race Data Table	117
2.1.1.5.	Stadium Data Table	117
2.1.1.6.	Relationships Between the Five Tables.....	119
2.1.2.	Data Cleaning and Quality Control.....	121
2.1.2.1.	Data Checking.....	121
2.1.2.2.	Greyhound Stadium (StadiumID)	123
2.1.2.3.	Number of Dogs in the Race (Ndogs).....	124
2.1.2.4.	Type of Race (RaceType)	124
2.1.2.5.	Race Finish Position (Rank).....	124
2.1.3.	Additional Calculations Performed on the Phenotypic and Performance . Raw Data	125
2.1.3.1.	Greyhound Racing Age (RacingAge).....	125
2.1.3.2.	Month-Year-Stadium Combination (MYStad)	125
2.1.3.3.	Type of Race (RaceType)	126
2.1.3.4.	Average Speed (Speed)	127
2.1.3.5.	Adjusted Rank Position (AdjRank).....	127
2.1.4.	The Final Data Set.....	128
2.1.5.	Creation of the Pedigree File for Genetic Analysis	129
2.1.6.	General Analysis and Statistical Methods.....	130
2.2.	Genome-Wide Association Study (GWAS) of Stress Fracture Injuries in the Racing Greyhound	131
2.2.1.	Case and Control Selection	131
2.2.1.1.	Sample Collection	132
2.2.1.2.	Muscle Tissue Sample Collection	133
2.2.1.3.	Skin Tissue Sample Collection	134
2.2.1.4.	Saliva Sample Collection.....	135
2.2.2.	Sample Processing.....	137
2.2.2.1.	DNA Extraction from Muscle Tissue Samples	137
2.2.2.2.	DNA Extraction from Skin Tissue Samples	139

2.2.2.3. DNA Extraction from Saliva Samples.....	140
2.2.3. Quantification of DNA.....	141
2.2.4. General Analysis and Statistical Methods.....	141
2.3. Salivary Cortisol and Heart Rate as Measures of Stress in the Racing Greyhound.....	143
2.3.1. Greyhound Selection.....	143
2.3.2. Sample Collection.....	144
2.3.2.1. Saliva Sample Collection.....	145
2.3.2.2. Heart Rate Measurement.....	148
2.3.2.3. Phenotypic Data	148
2.3.3. Cortisol Enzyme Immunoassay (EIA).....	149
2.3.4. General Analysis and Statistical Methods.....	151
Chapter Three.....	152
3. Investigation of Environmental Factors Affecting the Race Performance of British Greyhounds	153
3.1. Introduction.....	153
3.1.1. Environmental Factors Affecting the Race Performance of Greyhounds.	154
3.1.2. Environmental Factors Affecting the Race Performance of Thoroughbred Horses	157
3.2. Aims	157
3.3. Materials and Methods	158
3.3.1. Data Collection and Cleaning for Quality Control	158
3.3.2. Analysis of Non-Genetic (Environmental) Factors Affecting the Racing Performance of British Greyhounds.....	158
3.4. Results	161
3.4.1. Summary Statistics	161
3.4.2. Influence of Non-Genetic (Environmental) Factors on the Race Performance of British Greyhounds.....	165
3.4.2.1. Sex of the Greyhound (Sex).....	168
3.4.2.2. Greyhound Birth Year (BirthYrInd).....	170
3.4.2.3. Greyhound Racing Age (RacingAge).....	172
3.4.2.4. Number of Dogs in the Race (Ndogs).....	176
3.4.2.5. Starting Trap Number (Trap)	178
3.4.2.6. Greyhound Stadium (StadiumID)	181
3.4.2.7. Effect of Season and Stadium (MYStad).....	184
3.4.2.8. Type of Race (RaceType)	187

3.4.2.9. Distance of Race (RaceDist).....	187
3.5. Discussion	190
3.5.1. Sex of the Greyhound (Sex).....	190
3.5.2. Greyhound Birth Year (BirthYrInd)	193
3.5.3. Greyhound Racing Age (RacingAge)	194
3.5.4. Number of Dogs in the Race (Ndogs)	197
3.5.5. Starting Trap Number (Trap)	198
3.5.6. Greyhound Stadium (StadiumID)	201
3.5.7. Effect of Season and Stadium (MYStad).....	202
3.5.8. Race Distance (RaceDist) and Type of Race (RaceType)	204
3.5.9. Conclusions.....	205
Chapter Four.	207
4. Genetic Analysis of Race Performance in British Greyhounds.....	208
4.1. Introduction.....	208
4.1.1. Production of Greyhounds for the Racing Industry	208
4.1.2. Selection and Animal Breeding	209
4.1.3. Calculation of Estimated Breeding Value (EBV)	211
4.1.4. The Selection Index	214
4.1.5. Best Linear Unbiased Prediction (BLUP).....	215
4.1.5.1. The Linear Mixed Model.....	217
4.1.5.2. Estimation of Fixed Effects and Prediction of Random Effects.....	219
4.1.5.3. The Animal Model	220
4.1.6. Estimation of Variance Components.....	222
4.2. Aims	222
4.3. Materials and Methods	223
4.3.1. Data Collection and Cleaning for Quality Control	223
4.3.2. Influence of Environmental (Non-Genetic) Factors on the Race Performance of British Greyhounds.....	224
4.3.3. Animal Model Genetic Evaluations	224
4.3.3.1. Race Time	225
4.3.3.2. Speed	226
4.3.3.3. Rank	227

4.3.4. Estimation of Genetic Parameters and Breeding Values	228
4.3.4.1. Estimation of Genetic Parameters	229
4.3.4.2. Estimation of Breeding Values	230
4.4. Results	232
4.4.1. Estimation of Genetic Parameters	232
4.4.2. Estimation of Breeding Values	233
4.4.3. Genetic Trends.....	233
4.4.4. EBVs of Popular Greyhound Sires.....	238
4.4.5. EBVs of Popular Greyhound Dams	243
4.5. Discussion	248
4.5.1. Estimated Heritabilities of Greyhound Race Performance Traits	248
4.5.2. Repeatabilities of Greyhound Race Performance Traits	252
4.5.3. Estimated Breeding Values (EBVs) for British Racing Greyhounds	252
4.5.4. Phenotypic and Genetic Trends in Greyhound Race Performance.....	254
4.5.5. EBVs of Prolific British Greyhounds.....	255
4.5.5.1. EBVs of Prolific Greyhound Sires	256
4.5.5.2. EBVs of Prolific Greyhound Dams.....	257
4.5.6. Selection of Greyhounds for Breeding	258
4.5.7. Future Work.....	260
4.5.8. Conclusions.....	263
Chapter Five.	265
5. Genome-Wide Association Study of Stress Fracture Injuries in the Racing Greyhound.....	266
5.1. Introduction.....	266
5.1.1. Stress Fractures	266
5.1.1.1. Stress Fracture Injuries of the Racing Greyhound.....	266
5.1.1.2. Genetic and Environmental Risk Factors for Stress Fracture Injuries	268
5.1.2. Genome-Wide Association Studies (GWAS).....	270
5.1.2.1. Linkage Disequilibrium	271
5.1.2.2. GWAS Sample Sizes	271
5.2. Aims	273
5.3. Materials and Methods	273

5.3.1. Sample Collection and Preparation	273
5.3.2. DNA Extraction and Quantification	274
5.3.3. Selection of Case and Control Samples for GWAS	275
5.3.4. Illumina® Genotyping	277
5.3.5. Data Cleaning and Quality Control	279
5.3.6. Genome-Wide Association Analysis	281
5.4. Results	282
5.4.1. Creation of a DNA archive of British Racing Greyhounds	282
5.4.2. Genome-Wide Association Study	282
5.5. Discussion	297
5.5.1. Greyhound DNA Samples	297
5.5.2. Sample Collection and DNA Extraction	298
5.5.3. Single Nucleotide Polymorphisms (SNPs) and Chromosomal Regions of Interest	300
5.5.4. Limitations of the Study Design	308
5.5.5. Conclusions	310
Chapter Six.	312
6. Salivary Cortisol and Heart Rate as Measures of Stress in the Racing Greyhound.....	313
6.1. Introduction.....	313
6.1.1. Cortisol as a Measure of Stress	313
6.1.2. Measurement of Canine Cortisol	315
6.1.3. Transport of Greyhounds to the Race Track	316
6.2. Aims	318
6.3. Materials and Methods	319
6.3.1. Sample and Data Collection	319
6.3.2. Cortisol Enzyme Immunoassay (EIA)	320
6.3.3. Statistical Analysis	321
6.4. Results	322
6.4.1. Pre- and Post-Race Salivary Cortisol Concentration	322

6.4.2. Pre- and Post-Race Heart Rates	330
6.4.3. Correlation Between Salivary Log Cortisol Concentration and Heart Rate	336
6.4.4. Influence of Environmental Factors on Pre- and Post-Race Cortisol Concentration and Heart Rate	339
6.5. Discussion	353
6.5.1. Effect of Short Duration, High-Intensity Exercise on Salivary Cortisol Concentration and Heart Rate in the Racing Greyhound	353
6.5.2. Influence of Sex and Racing Age on Salivary Cortisol Concentration and Heart Rate in the Racing Greyhound.....	359
6.5.3. Influence of Race Distance on Salivary Cortisol Concentration and Heart Rate in the Racing Greyhound.....	359
6.5.4. Influence of Rank Finish Position as a Measure of Race Performance on Salivary Cortisol Concentration and Heart Rate in Racing Greyhounds ...	360
6.5.5. Influence of Travel Distance on Salivary Cortisol Concentration and Heart Rate in the Racing Greyhound.....	361
6.5.6. Conclusions.....	363
Chapter Seven.	367
7. General Discussion and Conclusions	368
7.1. Introduction.....	368
7.2. Aims	370
7.3. Overall Findings and Conclusions.....	371
7.3.1. Investigation of Environmental Factors Affecting the Race Performance of British Greyhounds	371
7.3.2. Genetic Analysis of Race Performance in British Greyhounds.....	376
7.3.3. Investigation of Genetic Factors in the Pathogenesis of Stress Fracture Injuries in the Racing Greyhound	380
7.3.4. Salivary Cortisol and Heart Rate as Measures of Stress in the Racing Greyhound.....	383
7.3.5. Conclusions.....	387
References	388

Appendices.....	459
Appendix 1: British Racing Greyhound Pedigree and Performance Database, Genetic Analysis and Estimated Breeding Values.	459
Appendix 2a: Details of the module 'Check_Dog_Table'	459
Appendix 2b: Details of the module 'Check_Pedigree_Table'	461
Appendix 2c: Details of the module 'Check_Performance_ID'	463
Appendix 3: The Structured Query Language - 'Check_Pedigree_Data' query	464
Appendix 4: Information sheet for Greyhound owners or trainers considering donation of a saliva sample from a dog.	465
Appendix 5: Information sheet for Greyhound owners or trainers considering donation of a tissue sample from a dog.	466
Appendix 6: Consent form for the donation of a saliva sample from a Greyhound.	468
Appendix 7: Consent form for the donation of a tissue sample from a Greyhound.	469
Appendix 8: Form used to obtain Greyhound phenotypic data from the owner or trainer donating a saliva sample for use in the Genome-Wide Association Study.....	470
Appendix 9: Phenotypic Data for Greyhounds in the GWA Study.	471
Appendix 10: Greyhound Phenotypic Data, Heart Rates and Cortisol	
Measurements.....	474
Appendix 11: Information sheet for Greyhound owners or trainers considering donation of a saliva sample from a dog for use in the study of salivary cortisol concentrations.....	480

List of Tables

Table 1.1. The total expenditure of the British Greyhound Racing Fund (BGRF) in the years 2013 to 2015, categorised into areas of welfare, regulation, prize money and commercial (marketing) expenditure (British Greyhound Racing Fund, 2014, 2015).	10
Table 1.2. Typical heritability (h^2) estimates for a number of traits of economic importance in various livestock species (Bourdon, 2014; Correa and Mota, 2007; Desgorces <i>et al.</i> , 2012; Ryan, 1975; Simm, 1998; Taubert <i>et al.</i> , 2007).....	67
Table 1.3. Heritability (h^2) estimates for a number of performance traits of economic importance in racing animals (Desgorces <i>et al.</i> , 2012; Ekiz and Kocak, 2005; Ekiz <i>et al.</i> , 2005a; Ryan, 1975; Taubert <i>et al.</i> , 2007; Thiruvankadan <i>et al.</i> , 2009a,b; Villela <i>et al.</i> , 2002).....	68
Table 2.1. Commonly used Greyhound running remarks recorded by officials at the race track regarding the progress and performance of the dog during the race, along with their definitions (Greyhound Racing Association Limited, 2014).....	114
Table 2.2. Preparation of Enzyme Immunoassay (EIA) substrate solution.....	151
Table 3.1. Number of race performance results (N), mean, standard deviation (SD), minimum (Min) and maximum (Max) values for the performance traits Race Time (s), Speed (m/s) and Rank (1 - 6) over a race distance of 480 m in British racing Greyhounds.	163
Table 3.2. Number of race performance results (N), mean, standard deviation (SD), minimum (Min) and maximum (Max) values for the performance traits Race Time (s), Speed (m/s) and Rank (1 - 6) in British racing Greyhounds over all race distances (220 m to 1,080 m).	163
Table 3.3. Number of race performance results (N), mean, standard deviation (SD), minimum (Min) and maximum (Max) values for the performance traits Race Time (s), Speed (m/s) and Rank (1 - 6) in British racing Greyhounds according to Race Type (1 - 4). Where: Race Type 1 = race distances \leq 305 m; Race Type 2 = race distances $>$ 305 m and $<$ 539 m; Race Type 3 = race distances \geq 540 m and \leq 700 m; Race Type 4 = race distances $>$ 700 m.	164

Table 3.4. Results of the univariable analyses of the influence of various environmental (non-genetic) variables on the performance measures Race Time (over a 480 m distance), Speed and Rank (over all distances). F Values and p - values are given. A grey coloured block indicates that the variable was not tested.	165
Table 3.5. Summary of all the environmental (non-genetic) variables tested and the statistical significance of results on race performance measures in British Greyhounds. Where: n.s. = not significant $p > 0.05$; * = significant $p < 0.0005$; ** = significant $p < 0.0001$. A grey coloured block indicates that the effect was not tested.	167
Table 4.1. The proportion of genes in common between Individual X and various classes of relatives.	213
Table 4.2. The additive genetic variance (σ^2_a), permanent environmental variance (σ^2_{pe}), residual variance (σ^2_e), estimated heritability (h^2) and repeatability (R) for Race Time (over a 480 m race distance), Speed and Rank (over all distances), in British racing Greyhounds. The standard error (SE) is given in brackets.	232
Table 4.3. Estimated Breeding Values for the 20 top-ranking Greyhound sires in the data set, with respect to numbers of offspring produced, for the performance traits Race Time, Speed and Rank. <i>DogID</i> is the unique Greyhound identification number (1 - 73,344); <i>No. Offspring</i> is the number of offspring produced by the sire to date, within the data set; <i>Sire Rank</i> is the rank of the dog according to number of offspring produced; <i>EBV</i> is the calculated EBV of the dog; <i>SE</i> is the standard error of the EBV, and <i>GR</i> is the genetic rank of the dog (1 - 73,344) according to their EBV for the trait.	239
Table 4.4. Estimated Breeding Values for the 20 top-ranking Greyhound dams in the data set, with respect to numbers of offspring produced, for the performance traits Race Time, Speed and Rank. <i>DogID</i> is the unique Greyhound identification number (1 - 73,344); <i>No. Offspring</i> is the number of offspring produced by the dam to date, within the data set; <i>Dam Rank</i> is the rank of the dog according to number of offspring produced; <i>EBV</i> is the calculated EBV of the dog; <i>SE</i> is the standard error of the EBV, and <i>GR</i> is the genetic rank of the dog (1 - 73,344) according to their EBV for the trait.	244

Table 5.1. The allocation of racing Greyhound DNA samples to 48 wells in a PCR plate for the Genome-Wide Association Study (GWAS). Case (affected) and control (unaffected) samples were allocated to alternate wells, with male (blue) and female (pink) samples mixed on an ad hoc basis.....	277
Table 5.2. The genome-wide association study (GWAS) data for each single nucleotide polymorphism (SNP) with minor (second most common) allele A1 and major (most common) allele A2 in case and control groups containing n individuals can be represented as a 2 x 2 contingency table of disease status by allele count.....	289
Table 5.3. Racing Greyhound stress fracture GWAS results: the top 50 most significantly associated SNPs ($p \leq 0.001$) from the GWAS analysis. Key: Rank = order of statistical significance [1 (lowest p -value) to 50 (highest p -value)]; SNP = Identity of SNP marker; Chr = Chromosome; Position = Physical location (base-pair) of the SNP based on the CanFam3.1 or CanFam2.0 canine genome assembly SNP map; A1 = Minor (second most common) allele name (based on whole sample); F_A = Frequency of minor allele in cases; F_U = Frequency of minor allele in controls; A2 = major (most common) allele name; CHISQ = Basic allelic test chi-square (1 degree of freedom); p -value = Asymptotic p -value for this test; OR = Estimated odds ratio for A1 (minor allele).	291
Table 5.4. Racing Greyhound stress fracture GWAS results: Single Nucleotide Polymorphisms (SNPs) associated with stress fracture at the $p \leq 0.0001$ significance level and their location on the chromosome. Where: Rank = overall order of statistical significance [1 (lowest p -value) to 11 (highest p -value)]; Chr = Chromosome; Location = physical location of the SNP on the chromosome, based on the CanFam3.1 or CanFam2.0 genome assembly.	302
Table 5.5. Racing Greyhound stress fracture GWAS results: Single Nucleotide Polymorphisms (SNPs) of interest associated with stress fracture at the $p \leq 0.001$ significance level and their location on the chromosome. Where: Rank = overall order of statistical significance [12 (lowest p -value) to 20 (highest p -value)]; Chr = Chromosome; Location = physical location of the SNP on the chromosome, based on the CanFam3.1 or CanFam2.0 genome assembly.	303

Table 6.1. ANOVA results [p -values, F -statistics and degrees of freedom (between groups, within groups)] of associations between Greyhound sex (male=1, female=2), race distance (265 m, 420 m or 470 m) or adjusted rank finish position (rank) (1 to 6) and pre-race or post-race salivary log cortisol concentration (ng/ml) or heart rate (beats per minute, bpm) in British racing Greyhounds. Each association was found to be not significant ($p > 0.05$).....339

List of Figures

Figure 1.1. The conformation of a Greyhound.....	3
Figure 1.2. An overview of skeletal anatomy of the canine distal thoracic limb. Adapted from Evans and de LaHunta, 2012.	27
Figure 1.3. An overview of skeletal anatomy of the canine distal pelvic limb. Adapted from Evans and de LaHunta, 2012.	28
Figure 1.4. Lateral aspect of the left tarsus of the dog. Adapted from Evans and de LaHunta, 2012.	32
Figure 1.5. Caudal aspect of the right tarsus and metatarsus of the dog. Adapted from Evans and de LaHunta, 2012.	33
Figure 1.6. Dorsal aspect of the left carpus and metacarpus of the dog. Adapted from Evans and de LaHunta, 2012.	38
Figure 1.7. Example of a Single Nucleotide Polymorphism (SNP) at two different nucleotide positions in a DNA sequence from two individuals.	82
Figure 1.8. Diagram of the secretion, metabolism and excretion of cortisol. Adapted from Mostl and Palme (2002).	94
Figure 2.1. An example of UK Greyhound race event information displayed on the Greyhound-Data website for the year 2008 (Greyhound-Data, 2014).	104
Figure 2.2. An example of information displayed on the Greyhound-Data (2014) website for a particular Greyhound race event in the UK.	106
Figure 2.3. An example of phenotypic data and pedigree information displayed on the Greyhound-Data (2014) website for an individual Greyhound.	108
Figure 2.4. Structure of a canine pedigree over six generations (Gen. I to Gen. VI).	109
Figure 2.5. An example of information displayed in the Dog Data table of the Microsoft Access Database.	111

Figure 2.6. An example of information displayed in the Pedigree Data table of the Microsoft Access Database.	112
Figure 2.7. An example of information displayed in the Performance Data table of the Microsoft Access Database.	116
Figure 2.8. An example of information displayed in the Race Data table of the Microsoft Access Database.	118
Figure 2.9. An example of information displayed in the Stadium Data table of the Microsoft Access Database.	119
Figure 2.10. Illustration of the five relational tables, Dog Data, Pedigree Data, Performance Data, Race Data and Stadium Data, in the Microsoft Access database. Variables are each defined in Chapters 2.1.1.1 to 2.1.1.5.	120
Figure 3.1. The distribution of number of race performance records per dog (n = 50,452) during a five-year period from 2008-2012, in British Greyhounds.	162
Figure 3.2. The mean monthly racing speeds (m/s) of male (n = 950,184 performance results) and female (n = 761,305 performance results) British racing Greyhounds over all race distances (220 m to 1,080 m) during a five-year period from 2008 to 2012.	169
Figure 3.3. The mean racing speed (m/s) of British Greyhounds (n = 1,711,489 performance results) over all race distances (220 m to 1,080 m) during a five-year period from 2008 to 2012, according to birth year of the dog. Error bars indicate the standard error of the mean.	171
Figure 3.4. The distribution of age of the dog in months at the time of race, per race start, in British Greyhounds (n = 1,711,489 performance results) over a five-year period from 2008 to 2012.	173
Figure 3.5. Mean race speed (m/s) with respect to racing age (months) in male (n = 950,184 performance results) and female (n = 761,305 performance results) British Greyhounds over all race distances (220 m to 1,080 m) during a five-year period from 2008 to 2012.	174

- Figure 3.6. Mean race time (s) over a 480 m race distance with respect to racing age (months) in male and female British Greyhounds (n = 481,882 performance results) during a five-year period from 2008 to 2012....175
- Figure 3.7. The mean racing speed (m/s) of British Greyhounds (n = 1,711,489 performance results) over all race distances (220 m to 1,080 m) during a five-year period from 2008 to 2012, according to number of dogs (4-6) in the race. Error bars indicate the standard error of the mean.177
- Figure 3.8. The mean racing speed (m/s) of British Greyhounds (n = 1,711,489 performance results) over all race distances (220 m to 1,080 m) during a five-year period from 2008 to 2012, according to starting trap number (1-6) in the race. Error bars indicate the standard error of the mean.179
- Figure 3.9. The mean rank finish position (1-6) of British racing Greyhounds (n = 1,711,489 performance results) over a five-year period from 2008 to 2012, according to starting trap number (1-6) in the race. A rank of one was awarded to the Greyhound finishing first, and a rank of six awarded to the Greyhound finishing last. Rank results were adjusted prior to analysis to take into account the number of dogs in a race. Error bars indicate the standard error of the mean.180
- Figure 3.10. The distribution of British Greyhound race performance results (n = 1,711,489) according to Greyhound stadium (1 - 26), over a five-year period from 2008 to 2012.182
- Figure 3.11. The mean racing speed (m/s) of British Greyhounds (n = 1,711,489 performance results) over all race distances (220 m to 1,080 m) during a five-year period from 2008 to 2012, according to race stadium (1 - 26). Error bars indicate the standard error of the mean.183
- Figure 3.12. The monthly phenotypic mean race times (s) for British racing Greyhounds over a race distance of 480 m (n = 481,882 performance results) during a five-year period from 2008 to 2012, and the monthly mean temperature (degrees Celsius) in the UK. Temperature data obtained from DUKES (2014).185

Figure 3.13. The monthly phenotypic mean speeds (m/s) for British racing Greyhounds, based on all performance results (n = 1,711,489) across all race distances (220 m to 1,080 m) in a five-year period from 2008 to 2012, and the monthly mean temperature (degrees Celsius) in the UK. Temperature data obtained from DUKES (2014).....	186
Figure 3.14. The mean racing speed (m/s) of British Greyhounds (n = 1,711,489 performance results) over all race distances (220 m to 1,080 m) during a five-year period from 2008 to 2012, according to race type. Where: Race Type 1 = race distances ≤ 305 m; Race Type 2 = race distances > 305 m and < 539 m; Race Type 3 = race distances ≥ 540 m and ≤ 700 m; Race Type 4 = race distances > 700 m. Error bars indicate the standard error of the mean.....	188
Figure 3.15. The mean racing speed (m/s) of British Greyhounds (n = 1,711,489 performance results) over a five-year period from 2008 to 2012, according to race distance (220 m to 1,080 m).	189
Figure 4.1. Mean Estimated Breeding Value (EBV) of British racing Greyhounds (n = 73,344) for the performance trait Race Time (over a 480 m race distance), according to birth year of the dog.....	235
Figure 4.2. Mean Estimated Breeding Value (EBV) of British racing Greyhounds (n = 73,344) for the performance trait Speed (over all race distances), according to birth year of the dog.	236
Figure 4.3. Mean Estimated Breeding Value (EBV) of British racing Greyhounds (n = 73,344) for the performance trait Rank (over all race distances), according to birth year of the dog.	237
Figure 4.4. Estimated Breeding Values (EBVs) for Race Time over a 480 m race distance of the top 20 British racing Greyhound sires according to number of offspring produced.....	240
Figure 4.5. Estimated Breeding Values (EBVs) for Speed of the top 20 British racing Greyhound sires according to number of offspring produced.	241
Figure 4.6. Estimated Breeding Values (EBVs) for Rank of the top 20 British racing Greyhound sires according to number of offspring produced.	242

Figure 4.7. Estimated Breeding Values (EBVs) for Race Time over a 480 metre race distance of the top 20 British racing Greyhound dams according to number of offspring produced.....	245
Figure 4.8. Estimated Breeding Values (EBVs) for Speed of the top 20 British racing Greyhound dams according to number of offspring produced.....	246
Figure 4.9. Estimated Breeding Values (EBVs) for Rank of the top 20 British racing Greyhound dams according to number of offspring produced.....	247
Figure 5.1. A quantile-quantile (Q-Q) plot of $-\log(\text{expected } p\text{-values})$ and $-\log(\text{Unadjusted observed } p\text{-values})$ for the Genome-Wide Association Study (GWAS) of stress fracture injuries in the racing Greyhound.....	284
Figure 5.2. A Manhattan Plot for racing Greyhounds following genome-wide association analysis. The blue and red horizontal lines indicate a significance threshold level of $p \leq 0.001$ and $p \leq 0.0001$, respectively, for SNPs (CanFam3.1) associated with stress fracture in racing Greyhounds. Each SNP is represented by its $-\log_{10} p\text{-value}$ and chromosomal location. Key: Chr = Chromosome.	287
Figure 5.3. Ensembl (CanFam2.0) chromosome view of SNP BICF2P1266953 at base pair position 100823429 on chromosome X.....	295
Figure 5.4. Ensembl (CanFam3.1) chromosome view of SNP BICF2P1136919 at base pair position 6030423 on chromosome 20.....	295
Figure 5.5. Ensembl (CanFam3.1) chromosome view of SNP BICF2P303083 at base pair position 6051952 on chromosome 20.....	296
Figure 5.6. Ensembl (CanFam3.1) chromosome view of SNP BICF2S23433818 at base pair position 11906685 on chromosome 27.	296
Figure 6.1. The distribution of pre-race (A) ($n = 49$) and post-race (B) ($n = 62$) Greyhound salivary cortisol concentration (ng/ml).....	324
Figure 6.2. The distribution of Greyhound salivary log cortisol concentration (ng/ml) measured pre-race ($n = 49$).....	325
Figure 6.3. The distribution of Greyhound salivary log cortisol concentration (ng/ml) measured post-race ($n = 62$).	326

Figure 6.4. The distribution of pre-race (A) (n = 49) and post-race (B) (n = 62) Greyhound salivary log cortisol concentration (ng/ml).	327
Figure 6.5. The paired pre-race and post-race salivary log cortisol concentration (ng/ml) for each Greyhound ID (1 to 67) (n = 47).	328
Figure 6.6. Difference in salivary log cortisol concentration (ng/ml) between pre- and post-race paired samples for each Greyhound (n = 47).	329
Figure 6.7. The distribution of Greyhound heart rate (beats per minute) measured pre-race (n = 65).	331
Figure 6.8. The distribution of Greyhound heart rate (beats per minute) measured post-race (n = 58).	332
Figure 6.9. The distribution of pre-race (A) (n = 65) and post-race (B) (n = 58) Greyhound heart rate (beats per minute).	333
Figure 6.10. Paired pre-race and post-race heart rate (beats per minute) for each Greyhound ID (1 to 67) (n = 58).	334
Figure 6.11. Heart Rate difference (beats per minute) between pre- and post-race paired measurements for each Greyhound (n = 58).	335
Figure 6.12. Scatter plot of Greyhound pre-race salivary log cortisol concentration (ng/ml) and heart rate (beats per minute) ($R^2 = 0.0098$).	337
Figure 6.13. Scatter plot of Greyhound post-race salivary log cortisol concentration (ng/ml) and heart rate (beats per minute) ($R^2 = 0.0104$).	338
Figure 6.14. Pre-race geometric mean Greyhound salivary cortisol concentration (ng/ml) with respect to sex (male or female) (n = 49). Error bars indicate the standard error of the mean.	341
Figure 6.15. Post-race geometric mean Greyhound salivary cortisol concentration (ng/ml) with respect to sex (male or female) (n = 61). Error bars indicate the standard error of the mean.	341
Figure 6.16. Pre-race mean Greyhound heart rate (beats per minute) with respect to sex (male or female) (n = 49). Error bars indicate the standard error of the mean.	342

Figure 6.17. Post-race mean Greyhound heart rate (beats per minute) with respect to sex (male or female) (n = 57). Error bars indicate the standard error of the mean.	342
Figure 6.18. Post-race geometric mean Greyhound salivary cortisol concentration (ng/ml) with respect to race distance (metres) (n = 61). Error bars indicate the standard error of the mean.	343
Figure 6.19. Post-race mean Greyhound heart rate (beats per minute) with respect to race distance (metres) (n = 58). Error bars indicate the standard error of the mean.	343
Figure 6.20. Post-race geometric mean salivary cortisol concentration (ng/ml) with respect to adjusted rank finish position (1-6) of the Greyhound (n = 59). Error bars indicate the standard error of the mean.	344
Figure 6.21. Post-race mean heart rate (beats per minute) with respect to adjusted rank finish position (1-6) of the Greyhound (n = 56). Error bars indicate the standard error of the mean.	344
Figure 6.22. Pre-race Greyhound salivary log cortisol concentration (ng/ml) with respect to distance (kilometres) travelled by motor vehicle to the stadium (n = 49) ($R^2 = 0.0368$).	345
Figure 6.23. Post-race Greyhound salivary log cortisol concentration (ng/ml) with respect to distance (kilometres) travelled by motor vehicle to the stadium (n = 62) ($R^2 = 0.0075$).	346
Figure 6.24. Pre-race Greyhound heart rate (bpm) with respect to distance (kilometres) travelled by motor vehicle to the stadium (n = 65) ($R^2 = 0.00005$).	347
Figure 6.25. Post-race Greyhound heart rate (bpm) with respect to distance (kilometres) travelled by motor vehicle to the stadium (n = 58) ($R^2 = 0.001$).	348
Figure 6.26. The mean pre-race salivary log cortisol concentration (ng/ml) with respect to racing age (months) of the Greyhound (n = 49) ($R^2 = 0.0071$).	349

Figure 6.27. The mean post-race salivary log cortisol concentration (ng/ml) with respect to racing age (months) of the Greyhound (n = 62) ($R^2 = 0.005$).	350
Figure 6.28. The mean pre-race heart rate (beats per minute) with respect to racing age (months) of the Greyhound (n = 65) ($R^2 = 0.005$).....	351
Figure 6.29. The mean post-race heart rate (beats per minute) with respect to racing age (months) of the Greyhound (n = 58) ($R^2 = 0.006$).....	352

List of Abbreviations

The following list contains the abbreviations used throughout the thesis:

A	Additive Genetic Effects
ABB	Association of British Bookmakers
ACB	Accessory Carpal Bone
AHT	Animal Health Trust
AI	Artificial Insemination
AI-REML	Average Information Algorithm for Restricted Maximum Likelihood
ANOVA	Analysis of Variance
APGAW	Associate Parliamentary Group for Animal Welfare
ATP6V1C2	ATPase, H ⁺ transporting, lysosomal 42kDa, V1 subunit C2
BAGRA	Breeding Advisory Group for Racing Animals
BAGS	Bookmakers Afternoon Greyhound Service
BGRB	British Greyhound Racing Board
BGRF	British Greyhound Racing Fund
BLUE	Best Linear Unbiased Estimator
BLUP	Best Linear Unbiased Prediction
BMD	Bone Mineral Density
BMP	Bone morphogenic protein
bp	Base pair(s)
bpm	Beats per minute
BVA	British Veterinary Association
°C	Degrees Celsius
CHD	Canine Hip Dysplasia
cm	Centimetre(s)
CNTN1	Contactin 1
Cov	Covariance
CT	Computed Tomography
CTB	Central Tarsal Bone
CXorf64	Chromosome-X open reading frame 64
C3orf83	Chromosome 3 open reading frame 83
df	Degrees of freedom
dH₂O	Distilled Water
DNA	Deoxyribonucleic Acid
DXA	Dual-energy X-ray Absorptiometry
E	Environment
EBV	Estimated Breeding Value
EIA	Enzyme Immunoassay
E_p	Permanent environmental effects
E_t	Temporary environmental effects
G	Genotype
g	G-force
g	Gram(s)
G_A	Additive genetic effects
GBGB	Greyhound Board of Great Britain

GEBV	Genomic Estimated Breeding Value
G_{NA}	Non-additive genetic effects
GRA	Greyhound Racing Association
GWA	Genome-Wide Association
GWAS	Genome-Wide Association Study
GXYLT1	Glucoside xylosyltransferase 1
h²	Heritability
HPA	Hypothalamic-Pituitary-Adrenal
HR	Heart Rate
HRP	Horseradish Peroxidase
IGB	Irish Greyhound Board
kb	Kilobase(s)
KC	The UK Kennel Club
kg	Kilogram(s)
km	Kilometre(s)
LD	Linkage Disequilibrium
m	Metre(s)
M	Molar
MC	Metacarpal
ME	Metabolisable Energy
μl	Microlitre(s)
ml	Millilitre(s)
mm	Millimetre(s)
MME	Mixed-model equations
m/s	Metre(s) per second
MSC	Mesenchymal stem cell
MT	Metatarsal
MYO16	Myosin XVI
n	Number
NA	Non-Additive Genetic Effects
ND	Normally Distributed
ng/ml	Nanograms per millilitre
NGRC	National Greyhound Racing Club
NSB	Non-Specific Binding
OIE	World Organisation for Animal Health
OR	Odds ratio
P	Phenotype
<i>p</i>	<i>p</i> -value
PBS	Phosphate Buffered Saline
PBV	Predicted Breeding Value
PCR	Polymerase chain reaction
PDZRN4	PDZ domain containing ring finger 4
PPARG	Peroxisome proliferator-activated receptor gamma
PP2A	Protein phosphatase 2A
PPP2R2D	Protein phosphatase 2, regulatory subunit B, delta
Q-Q	Quantile-quantile
RCPA	Racecourse Promoters Association

REML	Restricted Maximum Likelihood
RGT	Retired Greyhound Trust
RNF175	Ring finger protein 175
rpm	Rotations per Minute
RSPCA	Royal Society for the Prevention of Cruelty to Animals
RXR	Retinoid X receptor
s	Second(s)
SCS	Salimetrics® Children's Swabs
SD	Standard Deviation
SE	Standard Error
SFRP2	Canis lupus familiaris secreted frizzled-related protein 2
SNP	Single Nucleotide Polymorphism
SST	Swab Storage Tube
TCERG1L	Transcription elongation regulator 1-like
TGF-β	Transforming growth factor beta
TSEN2	tRNA-splicing endonuclease subunit
UK	United Kingdom
USA	United States of America
Var	Variance
VDR	Vitamin D Receptor
V-ATPase	Vacuolar ATPase
VO₂ max	Maximal Oxygen Consumption

Abstract

A Multifactorial Genetic Approach to Improving Welfare in the Racing Greyhound.

Ruth Jessica Dockerty

Greyhound racing is the third largest spectator sport in the UK. The Greyhound industry, however, produces a surplus of unwanted young dogs that do not make the grade to race. The thesis aimed to investigate the effects of genetic and environmental factors on the variation of race performance in British Greyhounds. Furthermore, the thesis aimed to investigate genetic factors involved in the pathogenesis of Greyhound stress fracture injuries, as well as cortisol and heart rate indicators of stress in the racing Greyhound.

A total of 1,711,489 race performance results and six-generation pedigree data were compiled for 50,452 Greyhounds competing at 26 British race tracks over a five-year period (2008 to 2012). Environmental (non-genetic) factors of sex, birth year, starting trap and month-year-stadium combination of the race were found to be significantly associated ($p < 0.0001$) with the performance traits Race Time (over a 480 metre distance), Speed and Rank (over all race distances). In addition, racing age was significantly associated with Race Time ($p < 0.0001$), Speed ($p < 0.0001$) and Rank ($p < 0.0005$). Stadium and number of dogs in the race were significantly associated ($p < 0.0001$) with both Race Time and Speed. Type of race and race distance were significantly associated ($p < 0.0001$) with Speed.

Univariate mixed animal-model genetic evaluations were performed. Estimated heritabilities were found to be moderate-high for Race Time (0.44), moderate for Speed (0.37) and low for Rank (0.02). Repeatabilities were moderate-high for Race Time (0.56) and Speed (0.52), and low for Rank (0.03). Breeding values were estimated for 73,344 British Greyhounds for each performance trait. Both phenotypic and genetic improvements were observed in Race Time and Speed of British Greyhounds during the study period. This indicates that the current method of breeding based on phenotypic performance is effective for these traits. The study identified, however, that targeted genetic selection based on EBVs would be considerably more efficient.

An archive of 237 British racing Greyhound DNA samples was established. In a Genome-Wide Association Study, single nucleotide polymorphism (SNP) genotyping of 21 case (stress fracture) and 24 control (uninjured) DNA samples was performed. No SNPs were found to reach genome-wide significance. The study, however, identified a number of nominally associated SNPs that may be implicated in the pathogenesis of Greyhound stress fracture, and that warrant further investigation. 11 SNPs were significantly associated at the $p \leq 0.0001$ level and a further 96 SNPs at the $p \leq 0.001$ level. Several of the most significantly associated polymorphisms were identified within or in close proximity to genes involved in bone metabolism pathways. Further work would be required to validate the associations by fine-mapping and targeted sequencing using a larger sample size.

Additionally, the study found that high-intensity sprint exercise in Greyhounds results in a significant increase in salivary cortisol concentration, a measure of stress, which is detectable immediately post-race. There were significant differences pre-race and post-race between each Greyhound's paired salivary log cortisol concentration ($p = 0.000004$) and paired heart rate ($p = 0.002$).

Genetic analysis of British Greyhound race performance, and genetic factors involved in Greyhound stress fracture injuries, have not previously been reported. The results of this work, along with further studies, can be used to inform future Greyhound breeding practices. The responsible use of targeted genetic selection within the industry would result in the production of stronger, better-performing dogs of predictable and superior genetic merit. Importantly, this may improve Greyhound welfare by minimising the number of surplus dogs produced.

Chapter One.

Introduction and Literature Review

1. Introduction and Literature Review

1.1. Greyhound Breed Characteristics

The Greyhound is a breed of hunting dog (*Canis lupus familiaris*) belonging to the sighthound group. All sighthounds, also known as gazehounds, hunt predominantly using sight and speed rather than scent and endurance. Greyhounds are elite athletes and the fastest of all dog breeds, capable of accelerating to speeds of up to 18 metres per second (m/s) in just a few seconds (s) (Dobson *et al.*, 1988). At maximum acceleration, a racing Greyhound is able to reach full speed within 30 metres (m) or six strides from the starting trap, and can maintain speeds of 18 m/s for the first 250 m of a race. The cheetah, capable of reaching speeds of 30 m/s over three to four strides from a standing start, is the only land mammal able to accelerate faster than a Greyhound over a short distance (Kohnke, 2005).

The Greyhound is physically designed for speed, with a slender yet muscular conformation; long head and neck; long and powerful limbs; deep chest; slightly arched loin; and extreme flexibility of the vertebral column (Figure 1.1) (Clarke, 1980; Genders, 1975; The Kennel Club, 2014).

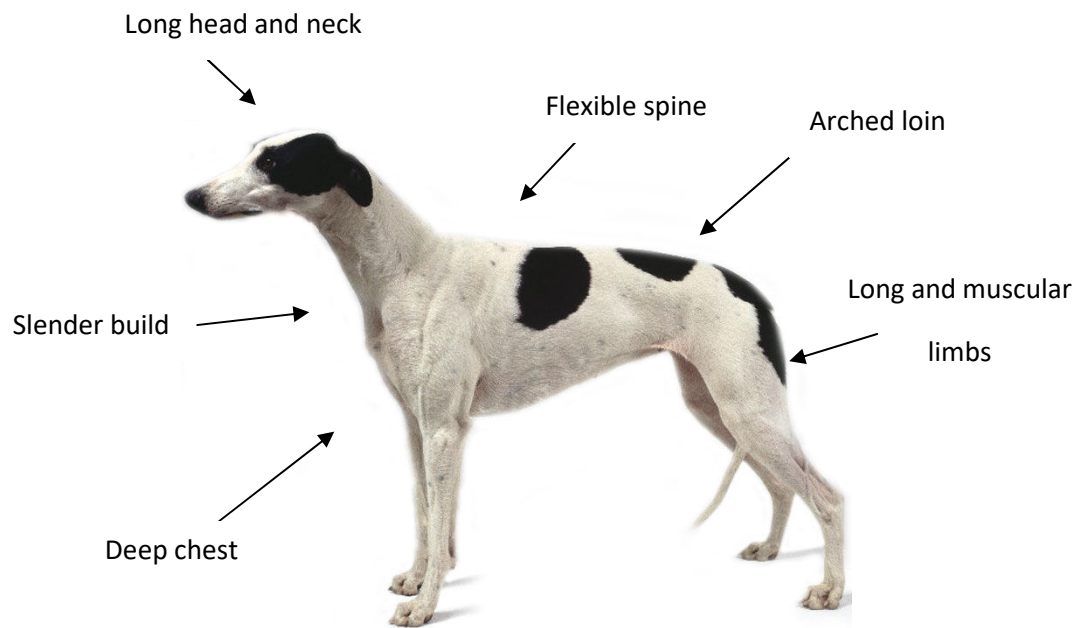


Figure 1.1. The conformation of a Greyhound.
(Photograph adapted from www.catsdogspictures.com, 2014)

Heart weight and heart-to-body weight ratios are higher in Greyhounds (1.25%) than crossbred dogs (0.80%) (Schneider *et al.*, 1964), and this is associated with racing ability (Schoning *et al.*, 1995). Greyhounds have greater muscle mass and less fat compared to other breeds of dog (Gunn, 1978; Hill *et al.*, 2005), with limb muscles containing 80% to 100% fast-twitch muscle type IIa fibres and very few slow-twitch fibres (Armstrong *et al.*, 1982; Gunn 1978). The fastest running gait of the Greyhound is described as a double suspension rotary gallop. This involves two support phases, and two flight phases in which all four feet are free from the ground, contracted and extended, during each full stride. The gallop can have either a right or a left lead (Brown, 1986).

Greyhounds have a fine and short coat, in a variety of colours. The UK Kennel Club specifies a breed standard describing the ideal physical appearance, characteristics and temperament of a show Greyhound (The Kennel Club, 2014). Within show lines, males range from approximately 71 centimetres (cm) to 76 cm in height at the withers, with weights varying from 27 kilograms (kg) to 40 kg, whereas females tend to be smaller and lighter, with heights of approximately 69 cm to 71 cm at the withers and weights ranging from 27 kg to 34 kg (The Kennel Club, 2014). Greyhounds bred for racing, however, are usually smaller and lighter than Greyhounds bred for the show ring (Blythe *et al.*, 2007; Lennox, 1987; The Kennel Club, 2016). The typical life expectancy of a Greyhound is between nine and 14 years, with a reported median longevity of 10.8 years (range 2.5 to 16.3 years) (Blythe *et al.*, 2007; O'Neill *et al.*, 2013).

Greyhounds have an innate desire to chase and catch prey, yet typically possess a gentle and even-tempered nature with their human companions (The Kennel Club, 2014). Variations in working intelligence and trainability have been reported for different breeds of dog (Ley *et al.*, 2009; Rooney and Bradshaw, 2004; Serpell and Hsu, 2005). While the Greyhound is considered an intelligent breed (The Kennel Club, 2014), hounds, including sighthounds, are reported to be less trainable than pointing or herding breeds (Ley *et al.*, 2009). The rearing environment and experiences of the dog during their first year of life, however, have been reported to influence their behaviour and temperament as adults, which may affect their trainability and suitability as working dogs (Foyer *et al.*, 2014). In addition, perceived differences in trainability between canine breeds or breed groups may

be a result of differences in physical characteristics rather than cognitive abilities (Helton, 2010).

1.2. History of the Greyhound

The Greyhound is reported to be one of the oldest canine breeds, with a history dating back over four thousand years to the Egyptians and Celts (Clarke, 1980; Genders, 1975). Pictures of similar smooth-coated sighthound-type dogs resembling the Saluki (Persian Greyhound) or Sloughi (North African sighthound) are depicted on ancient Egyptian tombs and ancient Greek vases (Davis, 1967). The exact origin of the Greyhound, however, remains unknown. Recent analysis of the genetic structure of the domestic dog has suggested that the Greyhound, and several other sighthounds including the Irish Wolfhound and Borzoi (Russian Wolfhound), are actually closely related to herding dog breeds such as the Border Collie, indicating that the Greyhound may be either an ancestor to or descendant of herding-type dogs (Parker *et al.*, 2004).

The Greyhound was first brought to the United Kingdom (UK) by traders in around 900 AD. They were initially used for hunting, and later for coursing and racing (Clarke, 1980; Genders, 1975; Lennox, 1987). All modern pedigree Greyhounds are descendants of dogs originally registered and recorded in 1882 in The Greyhound Stud Book (Greyhound Stud Book & National Coursing Club, 2014), which were later registered with the coursing, racing and kennel club authorities of the UK.

1.3. History of Greyhound Racing in the UK

Modern Greyhound racing has evolved from the sport 'coursing', in which Greyhounds are set to chase a live game animal, usually a hare, across open countryside (open-field coursing) or an enclosed area (closed-field coursing) (Stafford, 2007). Queen Elizabeth I introduced the first formal rules of hare coursing in the UK in the 1500s. These rules were still in use when the first official coursing club was formed in 1776 at Swaffham, Norfolk. The classic event of coursing was the Waterloo Cup, first held at Altcar near Liverpool in 1836, and it became a major national event (Greyhound Stud Book & National Coursing Club, 2014). Hare coursing has been illegal in the UK since February 2005, following the passage of the Hunting Act 2004 (England and Wales) and The Protection of Wild Mammals (Scotland) Act 2002. Coursing remains a popular sport in Ireland (Irish Coursing Club, 2014).

The first mechanical lure (an artificial hare or rabbit) and circular race track were invented in the USA in 1925 by Owen Patrick Smith. Smith joined with businessman Charles Munn, politician Brigadier-General Alfred Critchley and entrepreneur Sir William Gentle to promote Greyhound racing in the UK, and together they formed the Greyhound Racing Association (GRA). In 1926, the GRA built the first Greyhound stadium in the UK, Belle Vue Stadium in Manchester, which held its first race meeting on 24th July 1926. This marked the launch of commercial Greyhound racing in the UK (Clarke, 1980; Genders, 1975; Greyhound

Board of Great Britain, 2009; Lennox, 1987). The sport became very popular, and many other Greyhound stadiums were soon built throughout Britain. By the end of 1927, there were a total of 40 race tracks in operation (Greyhound Board of Great Britain, 2009).

Greyhound racing was most popular in the UK during the immediate post-Second World War period, with highest spectator attendances during this time. The sport, however, has experienced a decline in attendance from the early 1960s. This was partly due to the introduction of the 1960 Betting and Gaming Act permitting off-course cash betting, and the broadcasting of televised sports coverage. The introduction of online internet betting, and the 2005 Gambling Act enabling the evening opening of betting shops throughout the year, has led to a further reduction in spectator attendance (Greyhound Board of Great Britain, 2009). Total attendance figures have decreased from over 15 million in 1960 to 3.9 million in 2001 and 3.2 million in 2006. The number of spectators per licensed race meeting has decreased from over 2,000 in 1960 to approximately 600 in 2007 (Donoughue, 2007), and many Greyhound tracks have been forced to close. The number of licensed Greyhound race tracks in the UK has significantly decreased from 6,787 in 1960 (Donoughue, 2007) to 25 in 2015 (Greyhound Board of Great Britain, 2015), and the number of Independent UK tracks has decreased from 87 in 1960 (Donoughue, 2007) to 10 tracks in 2015. Greyhound racing, however, remains the third largest spectator sport in the UK. In addition, it is a popular off-course betting medium with over 2.5 billion pounds staked on the outcome of Greyhound races annually (Greyhound Board of Great Britain, 2009).

1.4. The Greyhound Racing Industry of the UK

The Greyhound racing industry of the UK is divided into two sectors: licensed Greyhound racing with a self-regulatory governing body; and Independent racing, also known as ‘flapping’.

1.4.1. Licensed Greyhound Racing in the UK

1.4.1.1. *Formation, Structure and Financing of the Licensed Greyhound Industry in the UK*

Prior to 1st January 2009, there were two main bodies responsible for the governance and regulation of the licensed Greyhound industry: The National Greyhound Racing Club (NGRC) and the British Greyhound Racing Board (BGRB). The NGRC was established in January 1928 as a non-profit-making regulatory body of the licensed industry, in order to develop and enforce a set of Rules of Racing. The BGRB was established in 1979, within the NGRC, as a forum to facilitate discussion between commercial stakeholders in the Greyhound industry regarding the promotion and improvement of the sport and Greyhound welfare. An additional role of the BGRB was to consult with the NGRC regarding the Rules of Racing (Greyhound Board of Great Britain, 2009).

Following an independent inquiry into the Greyhound racing industry in the UK (Donoghue, 2007), the NGRC and BGRB were abolished and replaced by a single

organisation to govern, regulate and manage licensed Greyhound racing: the Greyhound Board of Great Britain (GBGB), which became operational on 1st January 2009. There are 25 GBGB-licensed Greyhound race tracks in the UK to date; 24 in England and one in Scotland, all now regulated by the GBGB and subject to the GBGB Rules of Racing (Greyhound Board of Great Britain, 2014).

The British Greyhound Racing Fund (BGRF) is the official funding body for Greyhound racing in the UK, responsible for collecting voluntary contributions from licensed bookmakers and providing management and oversight of the GBGB budget (British Greyhound Racing Fund, 2012). The BGRF is a not-for-profit organisation that depends entirely on voluntary contributions. Bookmakers are requested to contribute 0.6% of their annual Greyhound racing turnover to the BGRF, under an agreement with the Association of British Bookmakers (ABB) (British Greyhound Racing Fund, 2012). The BGRF's total income from bookmakers voluntary contributions was 8.01 million pounds, 7.81 million pounds and 7.34 million pounds in the financial years 2012-13, 2013-14, and 2014-15, respectively (British Greyhound Racing Fund, 2014, 2015). The BGRF's total expenditure, categorised into areas of welfare, regulation, prize money and commercial (marketing), are displayed in Table 1.1 for the calendar years 2013 to 2015.

Area of Expenditure	Calendar Year		
	2013	2014	2015
Welfare	£3,263,000 (40.5%)	£2,887,300 (39.0%)	£2,909,190 (42.2%)
Regulation	£1,345,000 (16.7%)	£1,253,000 (17.0%)	£1,240,130 (18.0%)
Prize Money	£2,290,000 (28.4%)	£2,251,700 (30.4%)	£2,066,200 (29.9%)
Commercial	£1,152,000 (14.3%)	£1,008,000 (13.6%)	£684,480 (9.9%)
Total	£8,050,000	£7,400,000	£6,900,000

Table 1.1. The total expenditure of the British Greyhound Racing Fund (BGRF) in the years 2013 to 2015, categorised into areas of welfare, regulation, prize money and commercial (marketing) expenditure (British Greyhound Racing Fund, 2014, 2015).

Current areas of BGRF welfare expenditure include: welfare support grants to race stadiums to assist with funding the presence of veterinary surgeons at all race and trial meetings, donations to the Retired Greyhound Trust (RGT), welfare support for Greyhound trainers, welfare research projects, training and field-force welfare services (British Greyhound Racing Fund, 2014, 2015).

1.4.1.2. Greyhound Registration

Greyhound litters are 'earmarked' by an identification tattoo at 10 to 16 weeks of age, with the tattoo consisting of a unique combination of letters and/or numbers located on the medial ear pinnae. In order to qualify for registration in the GBGB Registry Database for racing at approximately 15 months of age, a Greyhound must already be registered by name in either the British or Irish Greyhound Stud Book. Additionally, each Greyhound is required to have a unique identification

microchip implant, with details registered on a national database. A Greyhound must be registered by and run in the true name of its Owner, who must also be registered with the GBGB. The registered Owner is responsible for the welfare of the Greyhound and for making suitable arrangements for the dog's retirement at the end of its racing career (Greyhound Board of Great Britain, 2014).

1.4.1.3. Greyhound Schooling

Young Greyhounds undergo a period of 'schooling', in which they are trained to chase an artificial lure, before starting their racing career at approximately 18 months of age. Schooling often involves galloping the pups in a long, straight exercise area behind a drag hare, before gradually introducing them to the elliptical race track and starting traps (Clarke, 1980; Lennox, 1987; Morris, 2009). The term 'Trial' is defined as any test run on a GBGB-licensed race track other than a Race. A Greyhound under 15 months of age is not permitted to run in any Race or Trial, with the exception of Schooling Trials (Greyhound Board of Great Britain, 2014).

1.4.1.4. Greyhound Training

Racing Greyhounds are trained by Professional Trainers, or non-professional trainers with a maximum of 12 Greyhounds, who must be licensed by the GBGB. The Greyhounds are required to live at their trainer's Licensed Kennel establishment, which must meet specified minimum requirements (Greyhound Board of Great Britain, 2014). Trainers are responsible for the feeding, exercise

and general daily care of Greyhounds residing at their Licensed Kennel. The training routine and race preparation for a Greyhound varies considerably between trainers and between dogs, but will usually involve a combination of walking, galloping, massage, rest periods and a carefully planned diet (Morris, 2009).

1.4.1.5. Greyhound Racing

In the UK, Greyhounds are raced in an anticlockwise direction around an elliptical track with a sand surface material. Stadiums vary widely in terms of track design, track length, radius of the bends and angles of inclination. Races, however, follow the same scheme on all GBGB-licensed race tracks. Male and female dogs are mixed and compete against each other, with a maximum of six dogs per race. Starting from automatic traps numbered from one (closest to the inside of the track) to six, the Greyhounds chase a mechanical lure that runs around the inner or outer circumference of the track. The first Greyhound to cross the finish line is declared the winner of the race. Each Greyhound stadium will usually hold two to three main race meetings per week all year round, with approximately 10 to 13 races per meeting. Additionally, Bookmakers Afternoon Greyhound Service (BAGS) meetings are currently held at 18 GBGB race tracks, to provide an extra programme of Greyhound races mainly to serve as an off-course betting medium (Greyhound Board of Great Britain, 2014).

There are strict rules and regulations regarding all aspects of racing and the licensed Greyhound Industry in the UK (Greyhound Board of Great Britain, 2014). Greyhounds can be run in either 'Graded' or 'Open' Races over a wide range of distances that vary both within and between race tracks. Approximately, the distance of a two-bend 'sprint' race ranges from 220 m to 305 m between tracks; four-bend 'middle-distance' races range from 375 m to 515 m; six-bend 'stayers' races range from 540 m to 700 m; and longer distance 'marathon' races are greater than 700 m. Racing may be flat or over hurdles, and may be handicapped or start from a level-break. The most common Greyhound racing distance in the UK is a 480 m flat race. Before taking part in a Graded race at a particular stadium, each Greyhound must first run between one and three Trials at that race track. Young dogs require three trials, the first of which must be a solo trial in which the dog runs by itself. Once the Greyhound has reached the required Grading Time, it is allocated the correct racing Grade by the Racing Manager and permitted to take part in a Race. A Graded dog will then race at the same track usually once a week. Dogs that have not raced for more than 28 days, or that are returning from injury, will be required to re-trial before racing.

In any one Graded Race, Greyhounds compete against other dogs of the same standard with equivalent race times. This is to ensure each dog has a fair chance of winning and to therefore increase the difficulty of betting. Two-bend 'sprint' races are graded from D1 (fastest) to D9 or D10 (slowest); four-bend 'middle-distance' races are graded from A1 (fastest) to A9 or A10 (slowest); six-bend 'stayers' races are graded from S1 (fastest) to S9 (slowest); and longer distance 'marathon' races

are graded from E1 (fastest) to E9 (slowest). A dog will be moved up or down the race grades if their performance improves or reduces, respectively. Any Greyhound can be entered into an 'Open Race' of any distance at any track, without the requirement for a Trial. Open Race events, however, usually represent the highest standard of Greyhound racing and are therefore aimed at the better-performing dogs. These Greyhounds often travel between tracks throughout the UK in order to compete in such events.

Greyhounds are often retired from racing when they reach three to four years of age. Some, however, will have shorter or longer racing careers than this, and there is no maximum age limit for a Greyhound to compete.

1.4.2. Independent Greyhound Racing in the UK

An Independent Greyhound stadium is defined as any commercial Greyhound race track in the UK that is not licensed and regulated by the GBGB. There are 10 Independent Greyhound tracks in the UK to date; six in England, three in Scotland and one in Wales. Greyhound racing at Independent tracks follows a similar format to GBGB-licensed racing. The rules of racing, however, are created and governed by the individual stadium and therefore vary considerably between tracks. In contrast to the licensed sector of the industry, there is no central database of Independent racing dogs, and Greyhounds may race under any name chosen by the owner.

1.5. Greyhound Racing Outside the UK

Greyhound racing is a popular sport worldwide, particularly in Ireland, the United States of America (USA), Australia and New Zealand. The Republic of Ireland has a very successful Greyhound industry, with significantly higher race attendance levels and prize money than the UK. From 1995 to 2007, attendances at race tracks in Ireland have increased from 586,000 to 1.3 million; prize money has increased from 2.4 million euros to over 12 million euros; bookmaker betting has risen from 22 million euros to over 90 million euros; and sponsorship increased from 610,000 euros to over 2.1 million euros (Irish Greyhound Board, 2007). During these years, over 90 million euros were invested in the sport's facilities by the Irish Greyhound Board (IGB or Bord na gCon), the regulatory body for Greyhound racing in the Republic of Ireland. There are 17 licensed Greyhound tracks in the Republic of Ireland to date, of which nine are owned by the IGB and eight are privately owned and operated. Additionally, there are two privately owned race tracks in Northern Ireland. There are no Independent or unlicensed Greyhound tracks in Ireland (Irish Greyhound Board, 2010).

The sport of Greyhound racing in Ireland is very similar to that in the UK. In the USA, Australia and New Zealand however, the standard number of participants in a Greyhound race is eight rather than six (Greyhound Racing Association of America, 2015; Greyhound Racing New South Wales, 2015; Greyhound Racing New Zealand, 2015).

1.6 Whippet Racing

The Whippet is a breed of hunting dog (*Canis lupus familiaris*) belonging to the sighthound group. Whippets are thought to have originated in England in the 17th Century, and are descendants of the Greyhound (The Kennel Club, 2014). The Whippet was officially recognised as a pure breed by the UK Kennel Club in the late 19th Century (The Whippet Club, 2016). The modern Whippet is smaller and lighter than the Greyhound, but otherwise similar in physical appearance and conformation. Height at the withers ranges from approximately 47 to 51 cm in male Whippets and 44 to 47 cm in females (The Kennel Club, 2014). The Whippet was originally bred for coursing and racing, and they are capable of running at speeds up to 16 m/s (American Kennel Club, 1998). Whippet racing was a popular sport in the UK during the 19th Century, particularly in the Midlands and northern areas of England (The Whippet Club, 2016). Pedigree Whippet racing remains a popular amateur sport in the UK, and is run by several racing clubs across the country. In contrast to Greyhound racing, betting on Whippet races is not permitted and there is no prize money (The Whippet Club, 2016).

1.7. Racing Injuries of the Greyhound

Greyhounds are at risk of developing injuries of varying cause and severity during their racing career, and sustain several specific musculoskeletal injuries that are relatively uncommon in other working or companion dogs (Davis, 1967; Hickman, 1975; Prole, 1976; Vaughan, 1969). The anatomy and conformation of a

Greyhound, along with the nature and consistency of high-speed racing in an anticlockwise direction around elliptical tracks, are considered to be the main reasons for the specific injuries they sustain (Davis, 1973; Prole, 1976). A combination of factors, however, are likely to contribute to the occurrence of racing injuries, including fitness of the dog; grade of race; speed; race distance; track design; degree of banking and radius of the bends; track surface material and conditions; track maintenance; and the weather (Cook, 1998; Davis, 1973; Hickman, 1975; Sicard *et al.*, 1999; Prole, 1976). Additionally, several studies have reported higher incidences of injuries in male racing Greyhounds compared to females (Davis, 1973; Gannon, 1972; Vaughan, 1969). The influence of genetic factors on the occurrence of racing injuries in Greyhounds, however, has not previously been reported.

The fastest running gait of the Greyhound consists of two support phases and two flight phases during each stride (Brown, 1986). Greyhounds demonstrate a circular sequence of foot landing, with the majority (84%) leading with their left thoracic limb when running in a straight line (Gillette and Zebas, 1991). During the front support phase, the foot of the left thoracic limb lands first followed by the foot of the right thoracic limb. This is followed by the front flight phase, in which all four feet of the Greyhound are free from the ground. In the rear support phase, the Greyhound then lands on the track with the foot of the right pelvic limb, closely followed by the foot of the left pelvic limb. The Greyhound then enters the rear flight phase, and once again all four feet are elevated from the track surface as the dog propels itself forward (Gillette and Zebas, 1991).

During the front support phase of the running gait, the force at which the foot of the leading limb impacts the ground is approximately 2.2 times the body weight of the Greyhound (Gillette, 1991). Consequently, in the majority of Greyhounds the left thoracic limb absorbs the largest impact during landing, and may be, therefore, more susceptible to injury than the subsequent landing limbs (Cook, 1998). The distribution of racing injuries between the limbs of British Greyhounds has been reported to closely follow the circular sequence of foot landing, with 40%, 29%, 19% and 12% of total injuries occurring in the left thoracic limb, right thoracic limb, right pelvic limb and left pelvic limb, respectively (Prole, 1976). Interestingly, Gillette and Zebas (1991) reported that 100% of Greyhounds observed lead with the right thoracic limb when navigating the bends of a race track, indicating that a change in footing takes place for the majority of Greyhounds as they approach a bend.

Greyhounds are subject to centrifugal forces as they travel anticlockwise around the bends of a track, and will lean to the left towards the inner rail of the bend in order to counteract the force and maintain their speed (Ireland, 1989). The lean of the Greyhound subjects the limb joints to horizontal forces, when they are only designed for considerable movement in the vertical plane (Gillette and Zebas, 1991). Gillette and Zebas (1991) calculated that for a racing Greyhound with a body weight of 32 kg, running at approximately 18 m/s around an unbanked bend with a 40 m radius, the limb joints would be subjected to a horizontal force of 25 kg. Increasing the radius of the turns, and increasing the surface gradient (banking)

around the bends of the track, would reduce the centrifugal forces acting on the Greyhound. This enables speed to be maintained at a reduced lean (Cook, 1998; Gillette and Zebas, 1991; Ireland, 1989), potentially reducing the risk of injury (Bloomberg, 1989, 1995). A surface gradient of 38 degrees would be required at the bend of the track in order to negate a horizontal force of 25 kg (Gillette and Zebas, 1991). This gradient, however, would be impractical to maintain using a sand surface material on a track that is approximately 6 m wide (Cook, 1998). A more manageable surface gradient of seven degrees at the bend of the track, for example, would reduce the horizontal force exerted on the limb joints of a Greyhound by approximately 16% compared to an unbanked bend (Gillette and Zebas, 1991).

In a survey of injuries at five Greyhound tracks over a two-year period in the state of Wisconsin, USA, Sicard *et al.* (1999) found that race distance, speed and track design had a significant effect on injury rate, with an increase in rate of injuries with successively higher grades of race. Injuries most commonly occurred at the first bend of a race, where speed is fast and there is high congestion of dogs. Greyhound body weight, ambient temperature, time of year, race number and starting trap position were found to have no significant effect on the rate of injuries. Sicard *et al.* (1999) reported that one Greyhound track had a significantly higher injury rate than the other four tracks in the study; this particular racecourse had several design differences in comparison to the other four, including a shorter straightaway section and smaller turning radius at the second bend.

Track surface conditions are likely to have an effect on the occurrence of racing injuries in Greyhounds (Cook, 1998), as reported for lameness in racehorses (Cheney *et al.*, 1973). The majority of Greyhound stadiums in the UK use sand materials at the track surface. Such tracks require regular watering in order to provide a cohesive surface on to which a Greyhound foot can grip (Cook, 1998). Inconsistencies in the track surface may result from poor track preparation or maintenance, over- or under-watering, uneven-watering or poor drainage (Cook, 1998). This may increase the risk of injury, as Greyhounds are not capable of safely adjusting to differing track surface conditions during racing (Davis, 1973; Gillette, 1992).

Greyhounds may sustain injuries during training, trialling or racing, however the majority of injuries occur during the course of a race (Prole, 1976). The most common injuries sustained are to the muscles, with reported incidences of 25% (Prole, 1976) and 26% (Sicard *et al.*, 1999) of total injuries in the UK and USA, respectively. Racing injuries involving the tarsus (hock) are also frequent, with reported incidences of 6% (Prole, 1976) and 25% (Sicard *et al.*, 1999) in the UK and USA, respectively. Tarsal injuries predominantly affect the right pelvic limb (Boudrieau *et al.*, 1984a; Gannon, 1972; Prole, 1976), most likely due to the stresses placed on this limb during anticlockwise racing (Anderson *et al.*, 1995). In a survey of racing injuries in the UK by Prole (1976), 98% of all injuries to the tarsus occurred in the right pelvic limb.

Racing injuries involving the carpus (wrist) are also common, with reported incidences of 11% (Prole, 1976) and 12% (Sicard *et al.*, 1999) of total injuries in the UK and USA, respectively. Approximately 66% of carpal injuries in Britain occur in the right thoracic limb (Prole, 1976). The lateral aspect of the right carpus, and the medial aspect of the left carpus, are the areas of the carpal joint most prone to injury in the British racing Greyhound (Guilliard, 1998, 2001; Guilliard and Mayo, 2000a,b, 2001). The Greyhound has to counteract centrifugal forces while travelling anticlockwise around a bend (Ireland, 1989). It is hypothesised that this results in increased tension on the side of the carpus closest to the outer perimeter of the track, potentially overloading the supporting joint structures (Guilliard, 1998, 2001; Guilliard and Mayo, 2000a,b, 2001).

Racing injuries involving the metacarpus or metatarsus are common, with reported incidences of 11% (Prole, 1976) and 7% (Sicard *et al.*, 1999) of total injuries in the UK and USA, respectively. In both the UK and Australia, approximately 75% of injuries to the metacarpus occur in the left thoracic limb (Gannon, 1972; Prole, 1976).

The incidence of toe injuries has considerably reduced following the change of track surface material in the UK from grass to sand (Poulter, 1981). In a survey of injuries at two grass tracks in London, Prole (1976) reported a 41% incidence of toe injuries. In comparison, injuries to the toes represented 13% of total injuries in a survey of five tracks in the state of Wisconsin, USA, with surfaces of sand or mixed sand and clay (Sicard *et al.*, 1999).

1.7.1. Muscular Injuries of the Racing Greyhound

Rapid acceleration of the Greyhound can exert massive strain on the skeletal muscles, and inadequate warm-up of the muscles prior to exercise is thought to be an important predisposing factor in the development of muscular injuries (Blythe *et al.*, 2007; Strickler *et al.*, 1990). Injuries to the skeletal muscles can be classified into four stages according to severity (Davis, 1973). Racing Greyhounds are prone to muscle ruptures, sprains, haematomas, splitting of muscle sheaths and generalised muscular pain, as well as several myopathies (Davis, 1967, 1973).

In the UK, the most commonly injured muscles are the triceps in the thoracic limb (Prole, 1976) and the gracilis muscle in the pelvic limb (Vaughan, 1969; Prole 1976), with both muscles being very prone to rupture (Sanders, 1962; Keene and Yarborough, 1966). Triceps injuries appear to have a similar prevalence in both right and left thoracic limbs of British (Prole, 1976) and Australian (Davis, 1973) racing Greyhounds. The majority of gracilis injuries, however, occur in the right pelvic limb of Greyhounds raced in the UK (Prole, 1968; Vaughan, 1969; Hickman, 1975). This is most likely due to stresses placed on the right pelvic limb during anticlockwise racing (Vaughan, 1969). Interestingly, a more even distribution of gracilis injury between right and left pelvic limbs has been reported in Australia (Davis, 1973). Ruptures of the triceps and gracilis muscles are usually accompanied by the development of a haematoma, resulting in distortion of the muscle contour at the lateral aspect of the shoulder and medial aspect of the thigh, respectively;

this is commonly known as a 'dropped muscle' (Vaughan, 1969; Davis, 1967; Hickman, 1975). Early treatment is required in order to minimise the amount of fibrous tissue formation at the rupture site, as this severely restricts locomotion in the limb and significantly reduces the racing potential of the dog (Davis, 1967; Hickman 1975). Injuries to the triceps and gracilis muscles do not seem to be associated with any specific actions or incidents, therefore the factors common to all Greyhound injuries are implicated (Hickman, 1975).

Additional muscles prone to racing injuries include the trapezius, infraspinatus, deltoideus and deep pectoral muscles in the thoracic limb; and the tensor fascia lata, biceps femoris, sartorius, vastus lateralis, rectus femoris and gluteal muscles in the pelvic limb (Davis, 1967, 1973).

In addition, Greyhounds may occasionally demonstrate a paralytic-type syndrome of muscle cramping during or after a race (Davis, 1967). This may be associated with dehydration, electrolyte imbalance or inadequate muscular warm-up prior to racing (Blythe *et al.*, 2007). Exertional rhabdomyolysis, also known as 'acidosis' or 'tying-up', is a potentially severe myopathy occasionally seen in racing Greyhounds (Davis, 1967, 1973) and other canine (Piercy *et al.*, 2001), equine (McGowen *et al.*, 2002) and human (Galvez *et al.*, 2008) athletes.

1.7.2. Tendon Injuries of the Racing Greyhound

Tendon injuries, ranging from sprains to full disruptions, are frequently observed in the racing Greyhound (Molyneux, 2005), with a reported incidence of 10% of total injuries in the UK (Prole, 1976). The digital flexor tendons of the thoracic limb are particularly prone to injury, representing 86% of all injuries to the metacarpus (Prole, 1976). As these tendons are located on the palmar surface of the metacarpal bones, they come into close contact with the ground through hyperextension of the carpus during racing, and are therefore prone to bruising and concussion injuries (Davis, 1967). The digital flexor tendons of digit five in the left thoracic limb and digit two of the right thoracic limb are most frequently affected (Molyneux, 2005; Prole, 1976). Overall, up to 75% of flexor tendon injuries occur in the left thoracic limb of British Greyhounds (Prole, 1976).

Other tendon injuries reported in the thoracic limb of the racing Greyhound include medial displacement of the tendon of origin of the biceps brachii muscle (Boemo and Eaton-Wells, 1995; Goring, 1984); rupture or avulsion of the biceps brachii and brachialis tendons of insertion (Schaaf *et al.*, 2009); and sprain or rupture of the flexor carpi ulnaris tendon at its insertion (Dee *et al.*, 1990; Prole, 1976). Sprain of the common calcaneal (Achilles) tendon, which is composed of the gastrocnemius tendon, superficial digital flexor tendon and the common tendon of the biceps femoris, gracilis and semitendinosus muscles (Evans and deLahunta, 2000), has been reported in the pelvic limb (Prole, 1976).

1.7.3. Ligament Injuries of the Racing Greyhound

Ligament injuries are commonly observed in the racing Greyhound (Prole, 1976), and can range from mild tearing to a complete disruption of the ligament fibres, or an avulsion fracture at the origin or insertion of the ligament (Roy and Dee, 1994). The majority of carpal injuries in the Greyhound are described as carpal ligament sprains, which most frequently occur in the right thoracic limb of Greyhounds raced in the UK (Prole, 1976). The forces exerted on the carpus during the thoracic limb weight-bearing phase of the gallop result in hyperextension of the carpal joint by up to 270 degrees, potentially causing overload of the supporting structures (Guilliard and Mayo, 2000b). In the pelvic limb of British Greyhounds, approximately 24% of injuries to the tarsus are considered to be tarsal sprains (Prole, 1976).

Specific ligament injuries reported in the racing Greyhound include sprains of the dorsal radiocarpal ligament (Guilliard, 1997); sprains or disorders of the short radial collateral ligament (Guilliard, 1998; Guilliard and Mayo, 2000a); avulsion fractures of the origins of the oblique and straight short radial collateral ligaments (Dee *et al.*, 1990); sprains or ruptures of the dorsal tarsal ligaments (Davis, 1983; Guilliard, 2003b); and rupture of the plantar tarsal ligaments (Dee *et al.*, 1990). Tears of the palmar superficial fascia over the accessory carpal bone (ACB) have also been described (Guilliard and Mayo, 2000b). In addition, degenerative changes of the support structures of the carpus and tarsus have been reported in

the racing Greyhound (Guilliard, 1998, 2005). In a radiographic survey of 100 racing or retired British Greyhounds, Guilliard (1998) found a 14% incidence of enthesiopathy (disorder of the muscular or ligamentous attachment to bone) of the origin of the straight part of the short radial collateral ligament, with a significant over-representation of the left carpus. The disorder, however, was not found to be of clinical significance, and there was no evidence of any adverse effects on performance.

1.7.4. Fractures and Luxations of the Racing Greyhound

Bone fractures and joint luxations/subluxations (dislocations) are common racing injuries of Greyhounds (Prole, 1976; Sicard *et al.*, 1999), with the majority occurring in the distal (lower) limb (Gannon, 1972). An overview of skeletal anatomy of the distal thoracic limb and distal pelvic limb are displayed in Figure 1.2 and Figure 1.3, respectively. Fractures of the central tarsal (CTB), fourth tarsal, calcaneus, accessory carpal (ACB), metacarpal (MC), metatarsal (MT) and phalangeal bones, and instability of the interphalangeal joints, are most frequently observed (Anderson *et al.*, 1995).

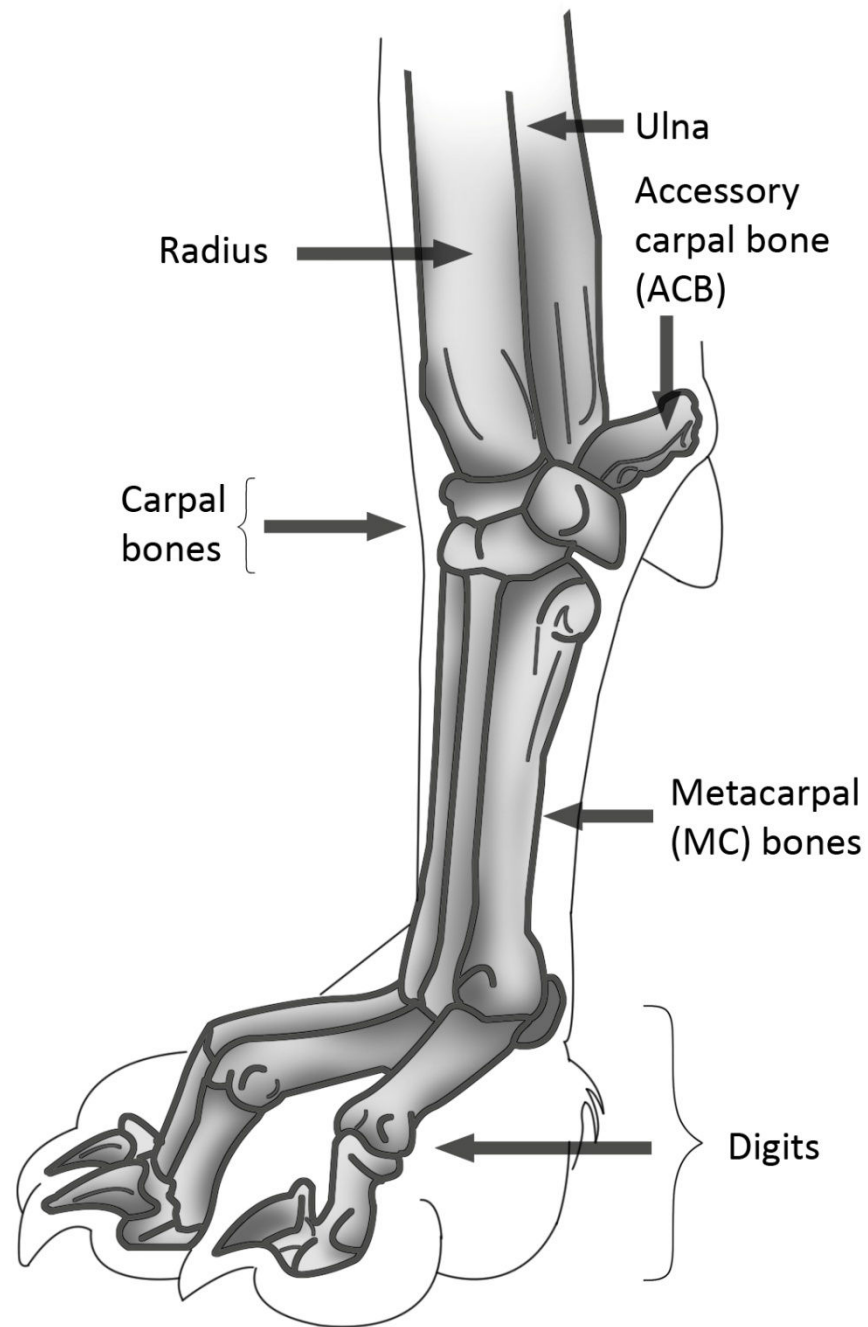


Figure 1.2. An overview of skeletal anatomy of the canine distal thoracic limb. Adapted from Evans and de LaHunta, 2012.

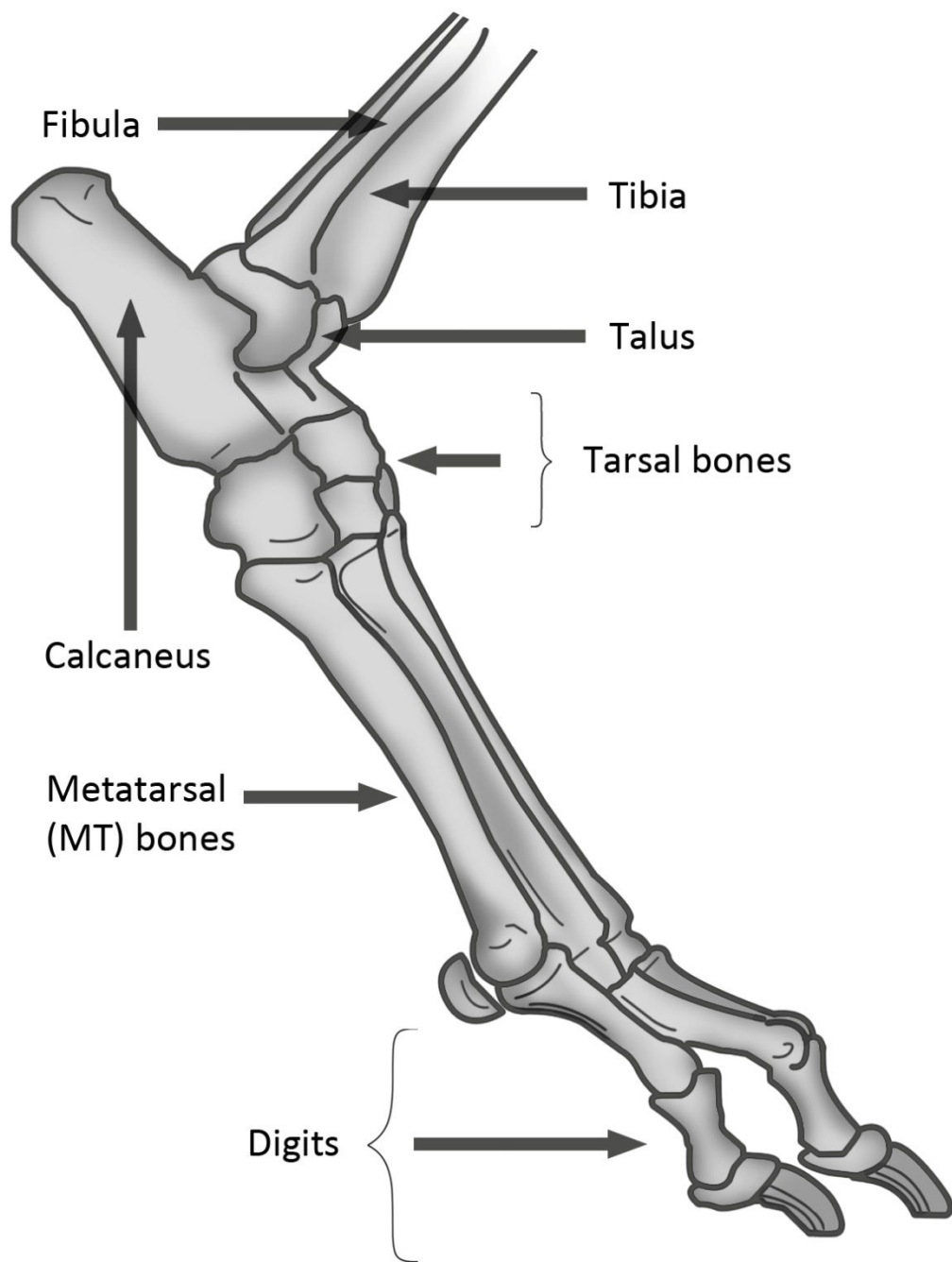


Figure 1.3. An overview of skeletal anatomy of the canine distal pelvic limb. Adapted from Evans and de LaHunta, 2012.

In a survey of racing injuries in the UK by Prole (1976), fractures and luxations have a reported incidence of 12% of total injuries; representing 9% and 21% of all injuries to the thoracic and pelvic limbs, respectively. In most domestic dog breeds, bone fractures are usually the result of external trauma or falling from a height (Gannon, 1972; Phillips, 1979). The majority of fractures that occur in racing Greyhounds, however, are not associated with direct trauma such as a fall or collision during racing (Siccard *et al.*, 1999). They are instead considered a result of bone fatigue, due to cyclic compressive loading causing an accumulation of micro-damage in the bone beyond its rate of repair by remodelling, until catastrophic fracture occurs through micro-crack dissemination or coalescence; such fractures are known as fatigue or stress fractures (Devas, 1961; Gannon, 1972; Taylor 1997, 1998).

In the racing Greyhound, stress fractures have been described in the central tarsal bone (CTB) (Bergh *et al.*, 2012; Boudrieau *et al.*, 1984a, 1984b; Devas, 1961; Emmerson *et al.*, 2000; Gannon, 1972; Johnson *et al.*, 2000; Muir *et al.*, 1999; Tomlin *et al.*, 2000), metacarpal (MC) and metatarsal (MT) bones (Bellenger *et al.*, 1981; Emmerson *et al.*, 2000; Gannon, 1972; Johnson *et al.*, 2001; Lipscomb *et al.*, 2001) and the acetabulum (Wendelburg *et al.*, 1988). Stress fractures are frequently observed in human athletes (Brukner *et al.*, 1996; Devas, 1969), racehorses (O'Sullivan and Lumsden, 2002, 2003) and in military recruits (Cline *et al.*, 1998; Kowal, 1980; Ross and Allsopp, 2002). While stress fractures are common injuries of the racing Greyhound, they are rarely seen in other breeds of dog (Emmerson *et al.*, 2000).

Fractures of the long bones (humerus, femur, radius, ulna, tibia and fibula) are relatively infrequent in racing Greyhounds, with reported incidences of 0.5% (Prole, 1976) and 2% (Siccard *et al.*, 1999) of total injuries in the UK and USA, respectively. Such fractures are usually associated with direct trauma such as a bump, collision or fall during the race (Prole, 1976; Siccard *et al.*, 1999), and are not associated with accumulation of bone fatigue micro-damage or branching micro-crack arrays (Tomlin *et al.*, 2000).

1.7.4.1. *Fractures and Luxations of the Carpus (Wrist)*

Racing Greyhounds are prone to fracture of the accessory carpal bone (ACB) (Davis, 1967; Dee and Dee, 1985; Gannon, 1972; Hickman, 1975; Prole, 1976); a short rod of bone located on the palmar aspect of the carpus (Figure 1.2). The ACB articulates with the ulnar carpal bone and styloid process of the ulna, and acts as a lever arm for several carpal flexor muscles (Evans and de LaHunta, 2000). In the racing Greyhound, the majority of ACB fractures occur in the right thoracic limb (Bateman, 1960; Johnson *et al.*, 1988; Prole, 1976). The injury is thought to occur as the Greyhound is travelling anticlockwise around a bend at high speed (Davis, 1967; Hickman, 1975), at the point when all of the dog's body weight is supported by the right thoracic limb (Bateman, 1960). The various types of ACB fracture have been described and classified into five categories based on their anatomic location and severity: types I to IV are avulsion fractures caused by excessive tensile loading of the ligaments or tendons inserting on the ACB, and type V fractures are

comminuted (Johnson, 1987). ACB fractures in the racing Greyhound do not respond well to conservative treatment, and surgery to remove the bone fragment is usually required (Bateman, 1960; Davis, 1963; Hickman, 1975). Luxation of the ACB (Guilliard, 2001) and subluxation of the second carpal bone (Guilliard and Mayo, 2001) have also been reported.

1.7.4.2. *Fractures and Luxations of the Tarsus (Hock)*

Fractures of the tarsal bones (Figure 1.4 and Figure 1.5) are commonly observed catastrophic injuries of the racing Greyhound, and account for approximately 67% of all injuries to the tarsus in the UK (Prole, 1976). The overwhelming majority of tarsal fractures occur in the right pelvic limb (Boudrieau *et al.*, 1984a; Davis, 1967; Devas, 1961; Gannon, 1972; Guilliard, 2000; Keene and Yarborough, 1966; Prole, 1976), most likely due to the stresses placed on the right tarsal joint during anticlockwise racing at high speeds (Anderson *et al.*, 1995).

The majority of Greyhound tarsal fractures involve fracture of the central tarsal bone (CTB) (Boudrieau *et al.*, 1984a; Gannon, 1972; Guilliard, 2000; Prole, 1976); an anatomically important bone located on the medial side of the tarsus (Figure 1.4 and Figure 1.5). The CTB articulates with each of the six other tarsal bones (Evans and de LaHunta, 2012).

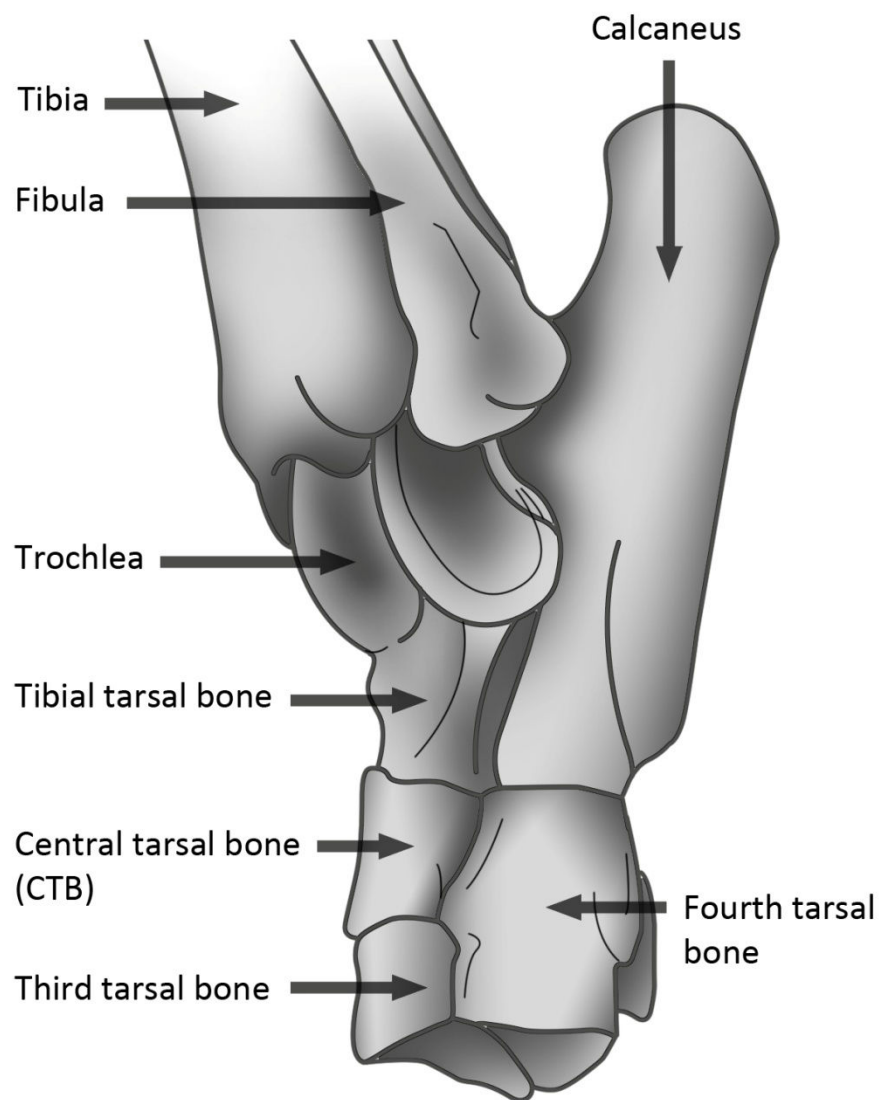


Figure 1.4. Lateral aspect of the left tarsus of the dog. Adapted from Evans and de LaHunta, 2012.

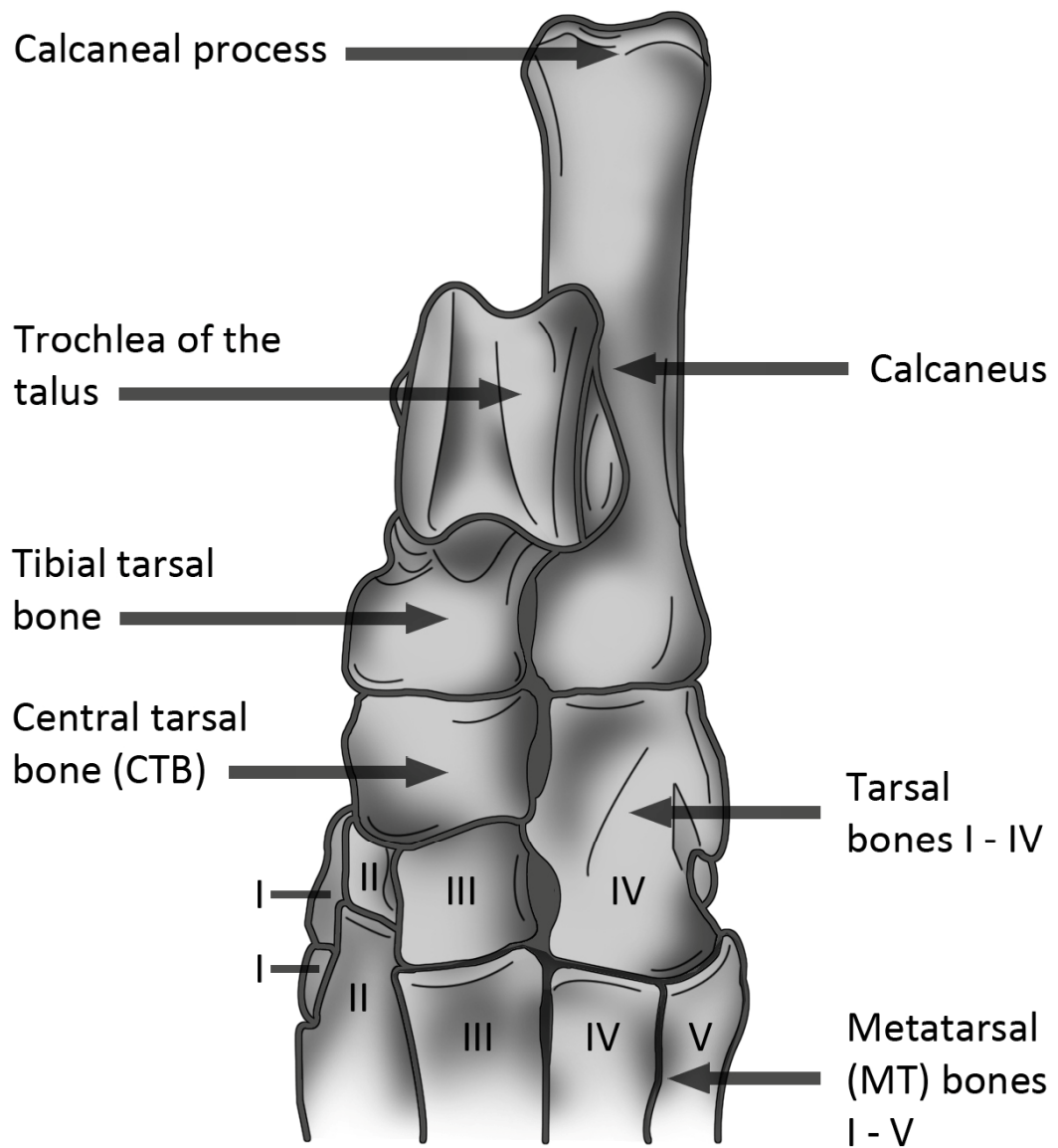


Figure 1.5. Caudal aspect of the right tarsus and metatarsus of the dog. Adapted from Evans and de LaHunta, 2012.

In the racing Greyhound, fractures of the CTB have a characteristic reproducible pattern and are considered stress fractures (Bergh *et al.*, 2012; Boudrieau *et al.*, 1984a, 1984b; Devas, 1961; Emmerson *et al.*, 2000; Gannon, 1972; Johnson *et al.*, 2000; Muir *et al.*, 1999; Tomlin *et al.*, 2000).

During anticlockwise racing around elliptical tracks, the CTB of the right pelvic limb is subjected to significantly greater compressive loads than the contralateral bone (Bergh *et al.*, 2012). Consequently, the right CTB demonstrates a greater adaptive re-modelling response, sustains a greater amount of matrix micro-damage, and has increased bone mineral density (BMD) compared to the left CTB (Johnson *et al.*, 2000; Muir *et al.*, 1999). As a result, up to 96% of CTB fractures in racing Greyhounds occur in the right pelvic limb (Boudrieau *et al.*, 1984a). The injury is usually sustained as the dog travels around a bend, when the compressive force placed on the medial aspect of the right tarsal joint is highest (Jones, 2009). Differences in BMD between fractured and intact CTBs of racing Greyhounds have been reported, with fractures occurring through a zone of increased BMD in the dorsal and mid-body regions of the right CTB (Bergh *et al.*, 2012). Stress fracture of the navicular (central) tarsal bone has been described in humans, and is associated with short-distance running in track athletes (Brukner *et al.*, 1996; Kiss *et al.*, 1993).

CTB fractures have been described and classified into five types based on radiographic assessment of the fracture configuration (Dee *et al.*, 1976): types I and II are dorsal slab fractures without or with displacement, respectively; type III

is a sagittal fracture with displacement; type IV fractures, involving a dorsal slab and medial bone fragment with displacement, are the most common with an incidence of 68% (Boudrieau *et al.*, 1984a); and type V fractures are severely comminuted and displaced. The introduction of an accurate classification system for CTB fractures led to improvements in surgical treatment, and reliable statistics on the likelihood of a successful return to racing (Boudrieau *et al.*, 1984b). A recent study by Hercok *et al.* (2011), however, found that computed tomography (CT) scans are superior to radiographs for the evaluation and classification of severe CTB fractures, with improved observer ability to accurately assess the fracture severity, degree of displacement, extent of comminution and identification of adjacent tarsal bone fractures.

Fracture of the CTB is one of the most common career-threatening injuries of the racing Greyhound (Jones, 2009). Dogs sustaining CTB fractures are often retired from racing or euthanased, mainly due to the financial costs of surgical treatment, the requirement for a long rest period and the possibility that the Greyhound may not regain its previous racing performance (Guilliard, 2000; Jones, 2009). In a study of 114 cases of CTB fracture in the USA, Boudrieau *et al.* (1984a) reported that 34% of Greyhounds sustaining a Type IV or V CTB fracture were euthanased or failed to return to successful racing. There is, however, a good prognosis for a return to competitive racing following surgical repair of the fracture (Boudrieau *et al.*, 1984b; Guilliard, 2000; Jones, 2009). In a study of 81 Greyhounds in the USA that underwent surgical repair of type III or IV CTB fractures, 88% successfully returned to racing (Boudrieau *et al.*, 1984b). Similarly, in a review of eight greyhounds in the

UK that underwent surgical repair of type III or IV CTB fractures, Guilliard (2000) reported that seven of the dogs returned to racing, with six of the dogs regularly achieving race times as good as, or better, than the pre-injury race times. For all types of CTB fracture, surgical repair by internal fixation gives superior results to conservative treatment by external coaptation (Boudrieau *et al.*, 1984b; Jones, 2009).

CTB fractures in racing Greyhounds are frequently associated with fractures of adjacent tarsal bones (Figure 1.4 and Figure 1.5) (Bellenger, 1981; Boudrieau *et al.*, 1984a; Emmerson *et al.*, 2000; Guilliard, 2000, 2010; Ost *et al.*, 1987; Prole, 1976). In a study of 114 cases of Greyhound CTB fractures in the USA, 64% of cases had additional fractures of adjacent tarsal bones; with the fourth tarsal bone, calcaneus and fifth metatarsal bone most commonly affected, in that order (Boudrieau *et al.*, 1984a). Fracture of the CTB with additional fracture of the third tarsal bone has also been reported (Guilliard, 2000, 2010). A survey of 113 Greyhound tarsal fractures in the UK by Guilliard (2010) found that 79% of cases involved CTB fractures, with concurrent fractures of adjacent tarsal bones in 61% of these cases. Additionally, a review of 51 Greyhound calcaneal fractures in the USA by Ost *et al.* (1987) found that 80% of cases were associated with fracture of the CTB. The prevalence of additional tarsal bone fractures appears to increase with the severity of the CTB fracture (Boudrieau *et al.*, 1984a).

It has been hypothesised that these secondary fractures may occur due to bone weakening from accumulated matrix micro-damage, or alternatively due to stresses that arise during collapse of the dorso-medial region of the tarsus at the moment of CTB fracture induction (Boudrieau *et al.*, 1984; Muir *et al.*, 1999). Isolated fractures of the tarsal bones without involvement of the CTB have also been described (Guilliard, 2010; Ost *et al.*, 1987). It has been suggested that many cases of isolated third tarsal bone fracture in British Greyhounds may be misdiagnosed as tarsal sprain injuries, due to the similarities in clinical signs and the fact that radiographic confirmation is often not obtained for Greyhounds racing in the UK (Guilliard, 2010).

Several hyperextension injuries of the tarsus have been reported in racing Greyhounds, including plantar ligament rupture resulting in proximal intertarsal joint luxation (Dee *et al.*, 1990); dorsal subluxations of the proximal intertarsal and the tarsometatarsal joints (Dee, 1998); and rupture of the dorsal tarsal ligaments resulting in dorsal tarsal instability (Guilliard, 2003b).

1.7.4.3. Fractures and Luxations of the Metacarpus and Metatarsus

Fractures of the metacarpal (MC) (Figure 1.2 and Figure 1.6) and metatarsal (MT) (Figure 1.3 and Figure 1.5) bones are common injuries of racing Greyhounds, with a high incidence observed in young Greyhounds between 14 and 28 months of age (Bellenger *et al.*, 1981; Gannon, 1972; Piras, 2005).

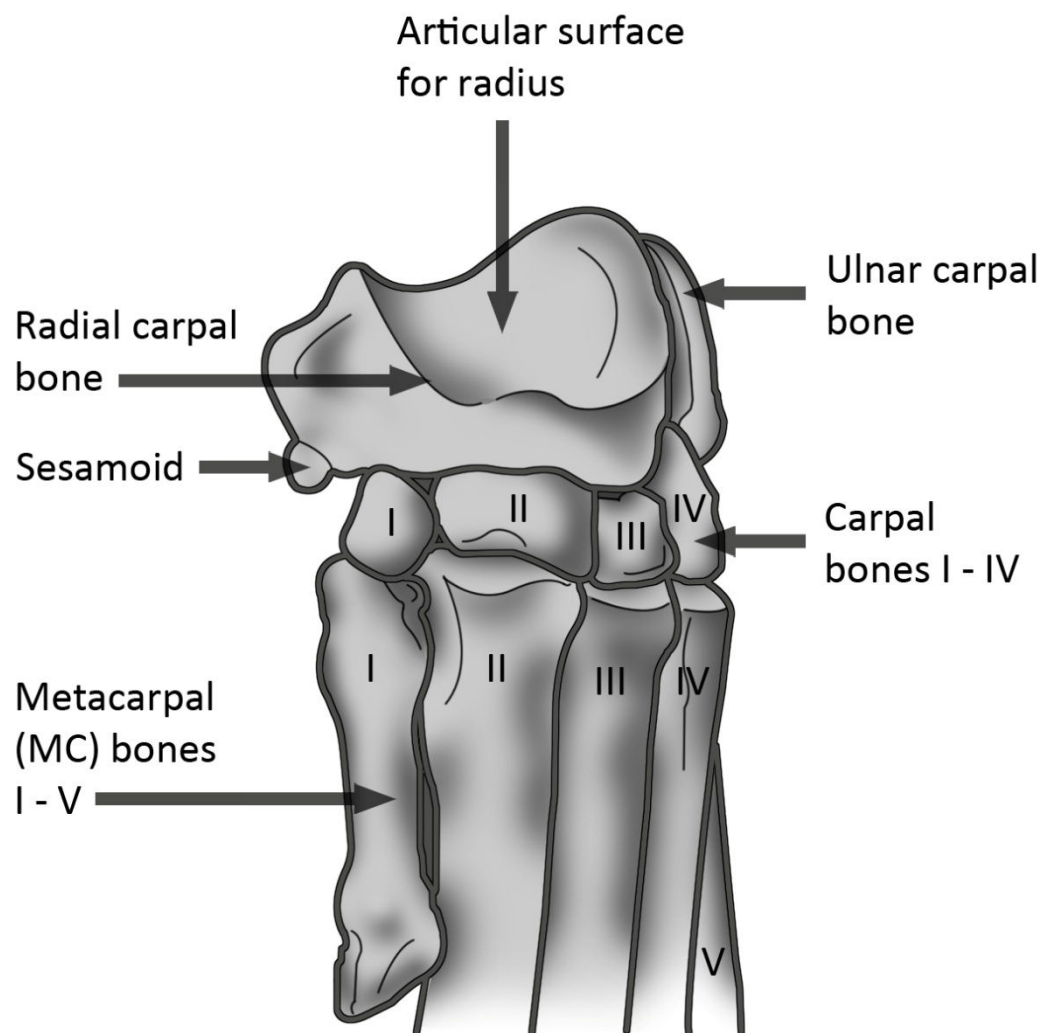


Figure 1.6. Dorsal aspect of the left carpus and metacarpus of the dog.
Adapted from Evans and de LaHunta, 2012.

Studies in Northern Ireland (Piras, 2005) and Australia (Gannon, 1972) have found that MC and MT fractures appear to be more frequent in male Greyhounds than females, potentially due to their greater body weight. Bellenger *et al.* (1981), however, reported a similar distribution of MC and MT fractures between male and female dogs racing in Australia.

In the racing Greyhound, fractures of the MC and MT bones are not usually associated with external trauma, and are instead considered stress fractures (Bellenger *et al.*, 1981; Emmerson *et al.*, 2000; Gannon, 1972; Johnson *et al.*, 2001; Lipscomb *et al.*, 2001). Similar stress fractures of the MC bones in racehorses (Devas, 1967; O'Sullivan and Lumsden, 2002) and the MT bones of human athletes (Brukner *et al.*, 1996; Devas, 1969) have also been described.

The majority of Greyhound MC fractures occur in the left thoracic limb, with the fifth MC bone (MC5) most frequently affected (Figure 1.6). This is thought to be due to transference of body weight onto the left thoracic limb as the Greyhound travels anticlockwise around a bend, resulting in increased loading of the MC bones closest to the inner circumference of the track (Bellenger *et al.*, 1981; Emmerson *et al.*, 2000; Gannon, 1972; Prole, 1976). Stress fractures of the left fourth (MC4), right second (MC2) and right third (MC3) MC bones are also commonly observed (Bellenger *et al.*, 1981; Gannon, 1972; Piras, 2005). In the pelvic limb, stress fractures of the left fifth (MT5) and the right third (MT3) (Figure 1.5) MT bones are most frequent (Piras, 2005).

Cyclic loading of the distal limb bones during anticlockwise racing initiates an asymmetric remodelling response in the MC and MT bones, particularly the left MC5 (Emmerson *et al.*, 2000; Johnson *et al.*, 2001). Measurement of bone mass in racing Greyhounds by use of dual-energy x-ray absorptiometry (DXA) has revealed large left-to-right differences in the bone mineral density (BMD) of MC and MT bones, with the greatest left-to-right difference demonstrated in MC5 (Emmerson *et al.*, 2000; Lipscomb *et al.*, 2001). Overall, a higher BMD has been detected in the bones closest to the inside of the track during racing, specifically the left MC5 (or MT5) and MC4 (or MT4), and the right MC2 (or MT2) and MC3 (or MT3) (Emmerson *et al.*, 2000). It is thought that the adaptive remodelling observed in certain distal limb bones of racing Greyhounds may be a protective biological response to limit the dissemination of micro-cracks, enabling the bone to sustain higher cyclic stresses and a greater amount of micro-damage before it ultimately fractures (Burr *et al.*, 1985; Mori and Burr, 1993; Johnson *et al.*, 2001, Lipscomb *et al.*, 2001).

1.7.4.4. *Fractures and Luxations of the Phalangeal Bones*

Fractures of the phalangeal bones (digits) (Davis, 1973; Gannon, 1972; Prole, 1976), interphalangeal joint luxations (Davis, 1967; Hickman, 1975) and sprains of the proximal interphalangeal joint (Guilliard, 2003a; Prole, 1976) are frequently observed injuries in racing Greyhounds, and most commonly occur in the left thoracic limb (Hickman, 1975; Prole, 1976). Gannon (1972) reported that phalangeal fractures in racing Greyhounds are not usually associated with direct trauma, and are likely to be stress fractures.

1.7.5. Miscellaneous Racing Injuries of the Greyhound

Other common racing injuries to the foot include torn or broken toe nails, foot or toe pad injuries, palmar (or plantar) skin lacerations and splitting of the webbing between the toes (Davis, 1967, 1973; Prole, 1976).

1.7.6. Genetic and Environmental Factors in the Pathogenesis of Stress Fractures

Greyhounds are prone to a variety of soft tissue and orthopaedic injuries during racing or training. The focus of this thesis is on stress fracture injuries of the racing Greyhound, as it is hypothesised that stress fracture is a multifactorial multigenic disorder resulting from the combined effects of both genetic and environmental factors (Yanovich *et al.*, 2012). Several risk factors have been identified in the development of stress fractures in human athletes and military recruits, including training intensity; tibia width; training surface; nutrition; smoking and motivation; female gender; increased age; type of physical activity; and poor fitness level at the start of a physical training program (Jones *et al.*, 2002; McClung and Karl, 2010). Furthermore, genetic risk factors have been implicated in the development of stress fracture injuries in humans (Chatzipapas *et al.*, 2009; Friedl *et al.*, 1992; Giladi *et al.*, 1986; Givon *et al.*, 2000; Milgrom *et al.*, 1985; Singer *et al.*, 1990). Investigation of the genetic factors involved in the pathogenesis of stress fracture in the racing Greyhound, however, has not previously been reported.

Stress fractures are common injuries of military personnel, with reported incidences of up to 5% of males and 21% of females (Jones *et al.*, 2002). In a study of male military recruits in Finland, Valimaki *et al.* (2005a) reported that tall height, poor physical fitness, high serum parathyroid hormone level and decreased bone mineral content and BMD were associated with increased risk of stress fracture.

Vitamin D is an essential nutrient that plays an important role in maintaining bone health. The binding of the biologically active metabolite 1,25-dihydroxyvitamin D [1,25(OH)₂D] to the vitamin D receptor enables maintenance of calcium homeostasis through the synthesis of calcibindin, which affects the efficiency of calcium absorption in the intestine (McClung and Karl, 2010). A reduction in levels of vitamin D causes secretion of parathyroid hormone, which may increase bone resorption. Several studies have investigated the relationship between vitamin D status, vitamin D supplementation, and the occurrence of stress fractures in humans (McClung and Karl, 2010). Ruohola *et al.* (2006) reported that a lower level of serum vitamin D concentration was a significant risk factor in the occurrence of stress fracture in Finnish male military recruits.

1.7.6.1. Genetic Factors in the Pathogenesis of Stress Fractures in Humans

There is indirect evidence suggesting the involvement of genetic factors in the pathogenesis of stress fracture in humans, including reports of identical stress fractures occurring at the same anatomical sites in human monozygotic twins (Singer *et al.*, 1990); multiple stress fractures occurring simultaneously in the same

individual (Milgrom *et al.*, 1985); and variation in stress fracture incidence between military recruits in the same unit undergoing similar training regimes (Giladi *et al.*, 1986). A study by Giladi *et al.* (1986) reported a high stress fracture recurrence rate; of all military recruits that sustained stress fractures during basic training, 10.6% developed subsequent stress fractures at a different anatomical site within one year. Givon *et al.* (2000) found a family history of stress fracture among first-degree relatives of 8.5% of Israeli Defence Force soldiers that developed high-grade stress fractures. In addition, Friedl *et al.* (1992) reported that a family history of stress fracture and/or other orthopaedic disorders occurred at a significantly higher rate amongst female military recruits that had sustained stress fractures. A family history of osteoporosis was associated with a ten-fold increased risk for stress fracture, suggesting that a genetic susceptibility is likely.

Several candidate gene association studies have been performed to investigate genetic factors in the development of stress fractures in human military recruits (Korvala *et al.*, 2010; McClung and Karl, 2010; Yanovich *et al.*, 2011, 2012). The genes coding for oestrogen receptors and androgen receptors have been proposed as candidate genes for regulation of bone mass and turnover (Valimaki *et al.*, 2005a). Valimaki *et al.* (2005b) compared the rates of *XbaI* and *PvuII* polymorphisms in the Oestrogen receptor alpha gene *ESR1*, and the CAG repeat polymorphism of the Androgen receptor gene, between 15 Finnish male military recruits with stress fractures (cases) and 164 without stress fractures (controls). No significant differences in polymorphisms between cases and controls were

observed. Other studies have investigated the link between genes involved in Vitamin D metabolism and the incidence of stress fractures in humans. Chatzipapas *et al.* (2009) compared the rates of four polymorphisms (*FokI*, *BsmI*, *ApaI* and *TaqI*) in the Vitamin D Receptor gene between 32 Greek military recruits with stress fractures and 32 controls. The authors reported that the *FokI* and *BsmI* polymorphisms were independent risk factors for stress fracture, associated with a 2.7-fold and 2.0-fold increased risk, respectively.

1.8. Recording and Reporting of Greyhound Injuries

Historically, Greyhound injury data has been routinely collected from many GBGB-licensed race tracks by the Racecourse Promoters Association (RCPA). At participating stadiums, track veterinary surgeons used standard injury reporting forms to record details of injuries sustained during a race meeting or trial session. These forms were submitted to the RCPA for entry onto a central database (APGAW, 2007). This injury data, however, was not usually made available to anyone except a few individuals within the RCPA and the Greyhound industry regulatory bodies (APGAW, 2007). On one occasion, the RCPA provided the BGRB with data collected from a total of 123,000 trials and races during a six-month period in 2006; in which the incidence of injury was 0.45%, with a relative incidence of 0.23% for injuries to the tarsus and carpus. These figures, however, only refer to severe injuries that result in a Greyhound being unable to race for a period of at least six weeks, and 'minor' injuries were not recorded. It is therefore

likely that a considerable number of injuries sustained by racing Greyhounds were not officially documented (APGAW, 2007).

Track veterinary surgeons typically record Greyhound injuries and treatment for their own clinical records. Currently, however, there is no publicly accessible database of injury data for racing Greyhounds in the UK. Injury data and statistics have never been published, neither for individual tracks nor the Greyhound industry as a whole, even on an anonymous basis or in summary form (APGAW, 2007).

In recent years, the GBGB have developed a Track Injury Database to act as a central repository for injury data collected from each of the GBGB-licensed stadiums (Greyhound Board of Great Britain, 2015). The track veterinary surgeon is required to enter injury records into the database within 24 hours of each race or trial meeting. Each stadium is provided with a summary of their respective injury statistics, along with the aggregated industry data for comparison, enabling areas of concern to be quickly identified.

1.9. Welfare of Racing Greyhounds in the UK

1.9.1 Definition of Animal Welfare

The World Organisation for Animal Health (OIE) (2016) defines animal welfare as how an animal is coping with the environment in which it lives. An alternative definition takes account of an animal's psychological state and feelings, and describes welfare as a broad term involving both the physical and mental well-being of an animal (Brambell Report, 1965). A third approach to welfare states that animals should be permitted to live according to their nature and be able to express their full range of behaviours (Kiley-Worthington, 1989). The most widely accepted definition of animal welfare combines all three areas, and describes welfare as the state of an animal's body and mind, and the extent to which its nature and normal behaviour are expressed (Duncan and Fraser, 1997; Hewson, 2003).

In 1965, the UK government commissioned an investigation into the welfare of animals in intensive livestock husbandry systems (Brambell Report, 1965). As a result, the Farm Animal Welfare Advisory Committee was established in 1967. The committee developed a set of guidelines recommending that animals require certain freedoms to ensure the best possible standards of welfare. The guidelines were first published in a press statement by the UK Farm Animal Welfare Council (1979), and became known internationally as the Five Freedoms (Farm Animal Welfare Council, 1979, 2009):

1. Freedom from thirst, hunger and malnutrition, by providing free access to water and a suitable diet.
2. Freedom from discomfort, by providing an environment appropriate to the animal's needs.
3. Freedom from pain, injury and disease, by prevention, rapid diagnosis and treatment.
4. Freedom to express normal behaviour, by providing sufficient space, suitable facilities and company of the animal's own kind where appropriate.
5. Freedom from fear and distress, by ensuring conditions and management that avoid psychological suffering.

The Farm Animal Welfare Council (2009) states that good welfare of an animal implies both physical fitness and a sense of well-being, and that any animal kept by man must at least be protected from unnecessary suffering. Although the Five Freedoms were originally intended for animals in livestock husbandry systems, they are now widely used as recommendations for ideal standards of welfare in all animals under human care, including companion, working, sport and laboratory animals, and animals kept in zoos, wildlife parks and sanctuaries (Farm Animal Welfare Council, 2009; World Organisation for Animal Health, 2016). The UK Animal Welfare Act 2006 came into force on 28th March 2007 in Wales and on 6th April in England, and places duty of care obligations on the owner or carer of an animal. Under the Act, any person responsible for an animal must legally take all reasonable measures to ensure that the needs of the animal, based on the defined Five Freedoms, are met to the extent required by good practice.

The original Five Freedoms have been criticised for focusing on the elimination of poor welfare and suffering (Farm Animal Welfare Council, 2009). The legal requirement in the Animal Welfare Act 2006 to provide for an animal's needs, however, implies a more positive approach to aim for good standards of welfare. In order to achieve high standards of welfare and a good quality of life, the Farm Animal Welfare Council (2009) state that an animal should, in addition, be provided with some of its wants, and that positive experiences must substantially outweigh the negative experiences over an animal's lifetime.

1.9.2. History of Welfare Concerns in the British Greyhound Racing Industry

Over the past decade, the welfare of racing Greyhounds in the UK has received greater media publicity and increased public and political interest. In July 2006, the Sunday Times newspaper published an article claiming that over a fifteen-year period, a builder's merchant in Seaham, County Durham, had killed up to 10,000 healthy ex-racing Greyhounds considered by their trainers to be no longer fast enough to race, and buried them at his property (Foggo, 2006). The article resulted in considerable public interest and concern over the welfare of racing Greyhounds and their fate following retirement from the sport. The issues raised by the article, and the proven destruction of at least two Greyhounds in this incident, led to the Associate Parliamentary Group for Animal Welfare (APGAW) initiating an inquiry in August 2006 into the welfare of racing Greyhounds in England (APGAW, 2007). In addition, the BGRB and NGRC jointly commissioned the Labour peer, Lord Bernard

Donoghue, to carry out an independent review of the Greyhound industry in the UK (Donoghue, 2007).

Welfare issues can be defined as matters that refer to evaluations of states concerning an animal's interests (Yeates, 2010). One of the main welfare issues surrounding racing Greyhounds in the UK, identified by both the APGAW inquiry (APGAW, 2007) and independent review of the industry (Donoghue, 2007), was the number of Greyhounds that seemed to 'go missing' either before, during or after their racing careers. There were large gaps in the existing Greyhound industry records concerning the numbers of dogs bred for, racing in and retiring from the sport; the numbers of young Greyhounds that failed to make it to the track; the numbers of retired Greyhounds that were kept as pets, rehomed or euthanased; details of what happens to the unwanted dogs; and the incidence of racing injuries (APGAW, 2007). Importantly, both the APGAW inquiry (APGAW, 2007) and independent review (Donoghue, 2007) highlighted that the Greyhound industry produces a surplus of unwanted young dogs each year that do not make the grade to race competitively.

Ireland has a very successful Greyhound industry, with a high demand for production of dogs for racing on Irish tracks. A large number of the Greyhounds bred in Ireland, however, are used to supply the British racing industry (APGAW, 2007; Donoghue, 2007). Young Greyhounds are frequently transported over from Ireland in vans and by ferry, for sale in the UK.

All Greyhounds intended for racing are earmarked at approximately 10 to 16 weeks of age. They are not registered with the racing regulatory body, however, until they begin their racing career at 15 to 20 months of age (Donoughue, 2007; APGAW, 2007). By comparing NGRC data for the numbers of Greyhounds earmarked and the numbers later registered for racing, the APGAW inquiry (2007) estimated that between 6,000 and 12,000 young Greyhounds bred to supply the British racing industry each year did not ever enter competitive racing, and were unaccounted for. It is likely that the majority of these Greyhounds initially entered training programmes, but were later discarded due to poor performance, behavioural problems or disinterest in racing (APGAW, 2007). Some of these young Greyhounds may have been retained as pets, rehomed, returned to Ireland or raced at Independent tracks. There was no accurate data available, however, to establish what happened to them, and it is likely that a considerable number may have been euthanased (APGAW, 2007).

It is commonly accepted that the process by which an animal dies is a welfare issue, in that it may involve pain or other suffering. Webster (1994, 2005), however, argues that death in itself (non-existence) is not a welfare issue, and that an animal does not have concerns over achieving longevity. In this case, the euthanasia of healthy unwanted young Greyhounds would not be considered a welfare issue, providing that euthanasia is performed humanely. Yeates (2010), however, argues that death may in fact be a welfare issue, in that it prevents the animal from experiencing relevant positive states. If an animal would be otherwise expected to have sufficient positive experiences and a life worth living, then death

can be considered as contradictory to the animal's interests as it involves an absence of these states. Similarly, if the presence of life would have negative value overall, for example due to incurable disease or intractable pain, then death could be considered a benefit as it excludes negative states (Yeates, 2010). From this perspective, the euthanasia of a healthy young Greyhound may be considered a welfare issue, as it involves ending the life of an animal that would otherwise be expected to have a life worth living.

In addition to young Greyhounds that fail to make it to the race track, an estimated 11,000 Greyhounds retire from licensed racing in the UK each year, often as a result of injury or a deterioration in performance. The Retired Greyhound Trust (RGT) is a national charity first established in 1975, with the aim of ensuring the long-term welfare of retired or unwanted racing Greyhounds (Retired Greyhound Trust, 2011). In addition to income generated through fundraising events and charitable donations, the RGT receives annual funding from the British Greyhound industry. In 2014, the RGT received approximately 1.8 million pounds through donations and gifts, a grant of 1.4 million pounds from the Greyhound industry through the British Greyhound Racing Fund (BGRF), £934,646 through branch income and a £53,635 donation from Retired Greyhound Events Limited (Retired Greyhound Trust, 2014). The RGT rehomes approximately 3,500 Greyhounds each year; an additional 1,500 Greyhounds are rehomed through other charities annually; an estimated 3,000 Greyhounds may be kept in kennels or retained as pets by their owners or trainers each year; an estimated 750 may be returned to Ireland; and the fate of the remaining 8,250 to 14,250 Greyhounds

each year is unknown (APGAW, 2007). There will be an additional unknown number of retired or unwanted dogs produced by the Independent sector of the Greyhound industry (APGAW, 2007). Consequently, the over-supply of young Greyhounds and the high numbers of dogs retiring from the sport each year result in the demand for rehoming exceeding the current provisions for retirement (Donoughue, 2007).

The reports of the APGAW inquiry (2007) and independent review of the British Greyhound industry (Donoughue, 2007) both recommend that urgent measures should be taken to address the significant welfare issue of over-breeding and over-supply of young Greyhounds for the sport. Whilst it would be difficult to restrict the numbers of Greyhounds bred and imported into the UK from Ireland, as this is likely to contravene European Union trade regulations, the Greyhound industry was advised to consider the standards of breeding and the traits for which Greyhounds are currently bred (APGAW, 2007; Donoughue, 2007). Recommendations were made for the licensing and inspection of breeding establishments, improved identification and tracking of greyhounds from birth to retirement, increased funding for retirement provisions and improvements in rehoming schemes. In addition, both the APGAW inquiry (2007) and independent review of the British Greyhound industry (Donoughue, 2007) recommend that further research into Greyhound breeding is required, in order to reduce the supply of unsuitable or injury-prone dogs.

1.9.3. Welfare Improvements in the British Greyhound Racing Industry

As a result of the recommendations made by Donoughue (2007), the licensed sector of Greyhound racing was required to undergo a major restructuring and reform process in order to maintain its self-regulatory framework. The NGRC and BGRB were abolished and replaced by a single body to govern, regulate and manage licensed Greyhound racing: the Greyhound Board of Great Britain (GBGB), which became operational on 1st January 2009 (Greyhound Board of Great Britain, 2009). The GBGB was required to obtain accreditation by the United Kingdom Accreditation Service (UKAS) in order to be considered acceptable as a transparent and auditable regulator of its own welfare standards. The GBGB now enforce a comprehensive set of rules and regulations applicable to all licensed Greyhound tracks, owners and trainers in the UK, in order to ensure that the sport meets specified welfare and integrity standards (Greyhound Board of Great Britain, 2014).

Over recent years, the licensed sector of the Greyhound industry has significantly increased the amount of funding it provides towards Greyhound welfare; from 2.66 million pounds in 2005 (British Greyhound Racing Fund, 2007) to approximately four million pounds, more than one third of its annual budget, in 2009 (Greyhound Board of Great Britain, 2009). The main areas of welfare expenditure include financial support for the RGT, veterinary attendance at race meetings and trial sessions, track safety improvements, track kennel

improvements, grants to trainers to improve their kennel and transport facilities, and welfare research (Greyhound Board of Great Britain, 2009).

In addition, the licensed racing industry has made many improvements in record-keeping and tracking of Greyhounds throughout their racing career. Traditionally, racing Greyhounds have been identified by means of ear pinnae tattoos (Chapter 1.4.1.2). There have been a number of reported cases, however, of Greyhounds having their ear pinnae mutilated or cut off so that they cannot be identified with a particular owner or trainer (APGAW, 2007). The traceability of Greyhounds from birth to retirement has been greatly improved by the requirement for a unique microchip to be implanted in all GBGB-registered Greyhounds before they take part in their first race or trial. In addition, the recent introduction of the Microchipping of Dogs (England) Regulations 2015 requires that all dogs of all breeds must be microchipped from eight weeks of age. The GBGB maintain details of all registered Greyhounds and their microchip numbers, owners and trainers on a central database. The microchips are scanned prior to each race to confirm the identification of the dog. Owners are required to inform the GBGB when a registered Greyhound has retired from racing or has died, and must give details of the cause of death (Greyhound Board of Great Britain, 2009). The requirement for microchipping may therefore improve Greyhound welfare by improving the traceability of the dog throughout its life, so that it is linked to a known owner who is accountable for its welfare. This does rely, however, on ensuring that records held in the central database are updated if the Greyhound is sold or rehomed. In addition, the implantation of a subcutaneous microchip does not guarantee that it

will remain in the dog for life. There have been reported cases of non-veterinary removal of microchips from Greyhounds, leaving the dog with an open wound (APGAW, 2007).

Furthermore, the Independent sector of the British Greyhound industry has taken part in a major reformation process over recent years. Prior to 6th April 2010, there was no requirement for licensing or regulation of Independent tracks, no code of practice or specified standards for Greyhound welfare, and no requirement for a veterinary surgeon to be present at race or trial meetings. Consequently, welfare standards at British Independent Greyhound tracks were reported to vary considerably (APGAW, 2007; Donoghue, 2007). Following the independent review (Donoghue, 2007) and APGAW inquiry (APGAW, 2007) into the Greyhound industry of the UK, the British Government introduced secondary legislation under the Animal Welfare Act 2006 in order to regulate the welfare of all racing Greyhounds, in both the licensed and Independent sectors of the industry. The aim was to establish a set of minimum welfare standards for all Greyhound tracks in England, and to improve the traceability of Greyhounds from birth to retirement.

The Welfare of Racing Greyhounds Regulations 2010 (England) came into force on 6th April 2010, and requires that all Independent Greyhound tracks in England must be licensed by their local authority. Additionally, all Greyhound tracks in England must: have a veterinary surgeon present at all race and trial meetings who inspects every Greyhound before it runs; provide appropriate veterinary facilities; keep

accurate records of all Greyhounds, owners, trainers and injuries; allow only Greyhounds that are microchipped to run in a race or trial; and, since April 2013, must also provide kennels for at least 20% of Greyhounds present at the track for racing or trialling.

The British Government and Greyhound racing industry have therefore taken considerable measures over recent years to improve the general welfare of racing Greyhounds in the UK, in both the regulated and Independent sectors of the sport. One of the most significant issues yet to be addressed, however, is the number of surplus young Greyhounds produced for and by the British Greyhound racing industry each year. Consequently, this thesis focuses on a potential solution to this welfare issue through the application of modern methods of genetic analysis to the selection and breeding of racing Greyhounds. Using racing speed as an example trait, the thesis investigates how the targeted genetic selection of Greyhounds for speed (in combination with other health, welfare and economically-important traits) would produce Greyhounds of superior and predictable genetic merit, and therefore reduce the overproduction of young dogs that do not make the grade to race.

1.10. Selection and Animal Breeding

A trait is defined as any visible or measurable feature of an individual (Bourdon, 2014). Certain animal traits, such as coat colour, are controlled by a single major gene and are inherited in a simple Mendelian fashion, in which the animals can be divided into discrete categories. Such characteristics are known as qualitative traits, and are usually controlled entirely by genes and therefore unaffected by non-genetic (environmental) influences (Simm, 1998). In animal breeding, however, the majority of traits of economic importance are controlled by many different genes at different loci, and are measured in units or as quantities on a continuous scale of performance. Such characteristics are known as quantitative traits; most of which are also affected by non-genetic (environmental) influences such as nutrition, management, training, climate, geographic location or exposure to disease (Simm, 1998).

Genetic improvement is dependent on the existence of genetic variation between individuals in a particular trait of interest. The objective of animal breeding is to accurately identify animals with the 'best' or 'superior' characteristics, and to select these individuals as parents of the next generation. Scientific advances in the twentieth century, particularly in the areas of population and quantitative genetics, statistics, reproduction, and more recently molecular genomics, have greatly improved the process of selective breeding. Following pioneering work in the early 1900s in areas of population and quantitative genetics by Ronald A.

Fisher, J. B. S. Haldane and Sewall Wright, these theories were first applied to animal breeding by Jay L. Lush in the 1930s (Falconer and Mackay, 1996; Simm, 1998). Over the past 50 to 60 years, scientific methods of selection based on performance measurements and genetic evaluations have become widely used in pig, poultry, sheep, dairy and beef cattle breeding. This has resulted in increased output, decreased costs and improvements in the quality of animal products (Simm, 1998). The resulting genetic improvement within the population is permanent.

1.10.1. Genetics of Quantitative Traits

The observed performance of an animal for a particular trait is known as its phenotype (P). For the majority of quantitative traits, the phenotype of an individual is dependent on its genotype (G) and the environment (E), which includes all non-genetic factors affecting its performance. This can be expressed as:

$$P = G + E \quad \text{(Falconer and Mackay, 1996)}$$

The genotype of an individual can be further divided into additive genetic effects (G_A), the combined effects of all alleles which act additively on a particular trait; and non-additive genetic effects (G_{NA}) due to dominance, where the effect of one allele at one locus can be influenced by the presence of another allele at that same locus, and epistasis, where the presence of an allele at one locus masks the effects

of another allele at a different locus. Additionally, the environmental influences affecting the phenotype of an individual can be further divided into permanent environmental effects (E_p) which affect the individual for life, and temporary environmental effects (E_t) which have a short-term effect on performance. This can be expressed as:

$$P = G_A + G_{NA} + E_p + E_t \quad (\text{Simm, 1998})$$

For the majority of quantitative traits, it is the additive genetic (G_A) part of the individual's genotype, also known as its breeding value, which is estimated and altered through selection. The effects of dominance and epistasis on quantitative characteristics are difficult to measure, and are usually considered as 'noise' in within-breed selection programmes (Simm, 1998).

For a population of animals, measuring the total amount of variation in performance for a trait of interest, and dividing this into both genetic and non-genetic effects, is an essential part of breed improvement programmes. The total phenotypic variance (σ^2_P) observed in a population of individuals, for a particular trait, can be divided into a component due to variation in the genotype of the individuals (genetic variance, σ^2_G), and a component due to variation in their environment (environmental variance, σ^2_E). This can be expressed as:

$$\sigma^2_P = \sigma^2_G + \sigma^2_E \quad (\text{Simm, 1998})$$

The genetic variance can be further divided into a component due to additive genetic variance (σ^2_{GA}), or the variance in breeding values, and variance due to the non-additive genetic effects (σ^2_{GNA}) of dominance and epistasis. In addition, the environmental variance can be further divided into permanent (σ^2_{Ep}) and temporary (σ^2_{Et}) environmental variation. This can be expressed as:

$$\sigma^2_P = \sigma^2_{GA} + \sigma^2_{GNA} + \sigma^2_{Ep} + \sigma^2_{Et} \quad (\text{Simm, 1998})$$

1.10.2. Phenotypic Selection

Traditional methods of animal breeding are based on phenotypic selection, in which selection decisions are based on performance information from the individual animal only. The pedigree of the animal, and performance of its relatives or offspring, are not considered (Bourdon, 2014). Selection based on phenotypic performance can result in genetic improvements within a breed or group of animals when the trait under selection is reasonably heritable, that is a large proportion of the observed phenotypic variation in the trait is due to genetic effects. Phenotypic selection, however, is a slow and inefficient method of animal improvement (Taubert *et al.*, 2007). As phenotypic expressions of traits are influenced by both genetic and non-genetic (environmental) factors, this selection system may not use the genetic potential of the population to maximum effect (Islam *et al.*, 2013).

1.10.3. Estimated Breeding Value (EBV)

Most modern animal breeding programmes aim to separate the effects of genotype and the environment, to enable the selection of animals that have high genetic merit to become parents of the next generation, rather than those that perform well as a result of favourable environmental conditions (Simm, 1998). It is not possible to know an animal's true breeding value for a particular trait. Modern methods of genetic analysis, however, can be used to quantify and adjust records of performance for known environmental influences, and calculate Estimated Breeding Values (EBVs), also known as Predicted Breeding Values (PBVs), for individual animals (Simm, 1998). The EBV estimates the proportion of superiority or inferiority in an individual's performance that is due to additive genetic effects that can be transmitted from parent to offspring (Bourdon, 2014; Simm, 1998). The EBV of an individual therefore indicates their genetic value for a particular trait. The calculation of EBVs for animals within a population group enables these individuals to be ranked according to their additive genetic merit, providing a useful tool for breeders in the selection of animals for breeding.

The EBV of an animal is obtained by measurement of phenotypic performance of the individual and/or its relatives for a particular trait, and that of the population group in which it performs. This requires a detailed performance recording system, accurate identification of individual animals and accurate pedigree records. The accuracy of EBV calculation for an individual animal can be increased by using

performance data from relatives and/or using repeated records of performance from the individual (Bourdon, 2014; Nicholas, 2003; Simm, 1998).

In traditional methods of EBV calculation, the performance records are usually first adjusted to take account of a number of environmental influences, such as age at measurement, season or geographic location, using one of several adjustment methods (Bourdon, 2014; Simm, 1998). One approach is to adjust records of performance using additive correction factors. This involves adding quantities to (or subtracting quantities from) the performance records of individuals belonging to particular groups, for example growth rate in different litter sizes at birth, to enable comparison between all individuals. Alternatively, multiplicative correction factors may be used. This method involves adjustment of performance records by multiplying by the correction factors for individuals belonging to particular groups. This method may be more appropriate than additive adjustments when the correction factors are intended to be used across population groups which demonstrate very different levels of performance. A third method involves standardising to adjust records of performance. The records from individuals born over a specified time period are allocated to a particular contemporary group, based on the factors to be adjusted for. Within each of these contemporary groups, the performance record of each individual is expressed as a deviation from the mean of the group, in standard deviation units. This enables the direct comparison of the standardised measurements across contemporary groups within a flock, herd or racing stable/kennel establishment (Simm, 1998).

An alternative approach involves calculation of EBVs using Best Linear Unbiased Prediction (BLUP), which is a statistical technique that enables the simultaneous estimation of environmental influences and prediction of EBVs. BLUP enables performance records of the individual and/or those of its relatives to be combined, taking into account the genetic relationships between them, in order to increase the accuracy of EBV prediction (Nicholas, 2003; Simm, 1998). BLUP models include equations that solve for contemporary group effects, which are the environmental factors common to all individuals within a contemporary group, by comparing the performance of relatives in different contemporary groups (Bourdon, 2014). BLUP procedures therefore account for both environmental and genetic variations between different contemporary groups during the calculation of EBVs. Consequently, this enables the direct comparison of the resulting EBVs across different contemporary groups, and across different flocks, herds or racing stable/kennel establishments where there are genetic links between them via related animals (Bourdon, 2014; Simm, 1998). BLUP is the method of choice for large-scale genetic evaluations, and is therefore the method selected for genetic evaluation of the British racing Greyhound population in this thesis.

EBVs are usually expressed in the same units as the record of performance, such as kilograms of live weight, or metres per second for speed (Simm, 1998). EBVs are usually expressed relative to the population group mean, which is set to zero, and can therefore have a positive or negative value or be equal to zero. The sign of the EBV (positive or negative) indicates whether the individual is expected to rank genetically above or below the mean of the population group on which the

calculations were performed (Simm, 1998). The current system in use by the UK Kennel Club (2014) is that EBVs for dogs take the same unit and direction as the original phenotype. For example, a lower race time would be favourable in racing Greyhounds, as the fastest dog will complete the race in the shortest amount of time. Consequently, more negative EBVs would be favourable for this trait. In this case, a negative EBV or positive EBV would indicate that the individual is expected to be genetically better (faster) or worse (slower) than the population group mean (set to zero), respectively.

The calculation of EBVs and their application to animal breeding programmes has been extremely successful in improving health, production, performance and trainability traits in many groups of animals worldwide, including sports horses, dairy and beef cattle, guide dogs, pigs, poultry, sheep and fish (Bourdon, 2014; Famula, 2012; Simm, 1998).

1.10.4. Heritability (h^2)

In order to calculate EBVs, an estimation of the heritability (h^2) of the trait being measured is required. Heritability is defined as the ratio of additive genetic variance (σ^2_{GA}) to phenotypic variance (σ^2_P):

$$h^2 = (\sigma^2_{GA}) / (\sigma^2_P) \quad (\text{Falconer and Mackay, 1996})$$

Heritability therefore represents the proportion of observed phenotypic variance in a trait that is due to additive genetic effects, and can be passed on to the next generation. The h^2 is expressed as a proportion from zero to one, or as a percentage from 0% to 100%, and is specific to a particular population (Willis, 1998). In general, traits with an estimated h^2 below 0.2, between 0.2 and 0.4, or greater than 0.4 are considered to be lowly heritable, moderate heritable or highly heritable, respectively (Bourdon, 2014). If the estimated h^2 of a particular trait is high, then a large proportion of the phenotypic variance in that trait is due to additive genetic effects, and it is expected that much of the superiority of the parents will be passed on to their offspring (Simm, 1998). If the estimated h^2 of a trait is low, then a large proportion of the phenotypic variation in the trait is due to non-genetic (environmental) effects. Consequently, the observed phenotypic performance of an individual is a poor indicator of its underlying breeding value for this trait, and selection based on phenotypic performance will be ineffective (Bourdon, 2014).

Estimation of h^2 is based on measuring the degree of similarity in performance between relatives (Falconer and Mackay, 1996; Nicholas, 2003; Simm, 1998), for example the regression of offspring performance on parent performance for a particular trait. More sophisticated techniques involve measuring the degree of similarity in performance between different classes of relative. This can be achieved using modern statistical methods, which simultaneously calculate the additive genetic relationship between all relatives in that population group.

There are differences in published values for h^2 both between and within traits. In general, fertility and survivability-related traits tend to have low h^2 . Production and performance traits such as speed, growth rate, milk or egg production tend to have moderate h^2 , and carcass and conformation-related traits tend to have the highest h^2 (Bourdon, 2014). Typical h^2 estimates for a number of traits of economic importance in various livestock species are displayed in Table 1.2. Heritability estimates for several performance traits of economic importance in racing animals are displayed in Table 1.3. There is considerable variation in the reported h^2 estimates for performance traits in horses (Ekiz and Kocak, 2005; Ekiz *et al.*, 2005a; Thiruvankadan *et al.*, 2009a,b; Villela *et al.*, 2002). This may be explained by the differences in approach and study design between studies, differences in the statistical method used for h^2 estimation and the inclusion of different environmental (non-genetic) factors in the genetic analyses. Failure to account for important environmental factors in the genetic analysis may result in an underestimation of the proportion of additive genetic variance associated with the trait.

Species	Trait	h^2 Estimate
Cattle (Dairy)	Milk yield	0.25 - 0.40
	Milk fat %	0.50 - 0.60
	Milk protein %	0.50 - 0.60
	Rear leg set	0.15
	Stature	0.50
	Teat placement	0.30
	Udder support	0.20
	Various fertility measures	0.01 - 0.05
Cattle (Beef)	Back fat thickness	0.40 - 0.50
	Birth weight	0.25 - 0.40
	Calving ease	0.05 - 0.15
	Calving interval	0.05
	Feed Conversion	0.40
	Mature weight	0.65
	Weaning weight	0.20 - 0.30
	Yearling weight	0.30 - 0.40
Sheep	Birth weight	0.10 - 0.30
	Carcass fat depth	0.25 - 0.35
	Fleece diameter	0.45 - 0.55
	Fleece weight	0.30 - 0.40
	Number of lambs born	0.15
	Weaning weight	0.15 - 0.25
	Yearling weight	0.20 - 0.25

Table 1.2. Typical heritability (h^2) estimates for a number of traits of economic importance in various livestock species (Bourdon, 2014; Correa and Mota, 2007; Desgorces *et al.*, 2012; Ryan, 1975; Simm, 1998; Taubert *et al.*, 2007).

Species/Type	Trait	h^2 Estimate
Dog (Racing Greyhound)	Race time (480 m race distance)	0.23 - 0.31
	Speed	0.18
	Rank at finish	0.10
Horse (Thoroughbred)	Race time (final time/average time)	0.06 - 0.84
	Race time (best time)	0.10 - 0.77
	Rank at finish	0.04 - 0.37
	Earnings/annual earnings	0.04 - 0.19
	Log earnings/log annual earnings	0.08 - 0.60
	Average earnings per start	0.02 - 0.30
	Log average earnings per start	0.34 - 0.56
	Handicap weight/timeform rating	0.17 - 0.93
Horse (Arabian)	Race time (final time)	0.18 - 0.30
	Race time (best time)	0.281
	Rank at finish	0.07
	Annual earnings	0.14
	Log annual earnings	0.15
	Earnings per start	0.17
	Log earnings per start	0.17
Horse (Trotter)	Race time (final time)	0.005 - 0.92
	Race time (average time)	0.23 - 0.74
	Race time (best time)	0.00 - 0.88
	Rank at finish	0.12 - 0.25
	Total earnings	0.00 - 0.75
	Log total earnings	0.00 - 0.46
	Average earnings per start	0.09 - 0.34
Horse (Quarter horse)	Race Time	0.17 - 0.41
	Speed Index	0.14 - 0.19
	Rank at finish	0.13

Table 1.3. Heritability (h^2) estimates for a number of performance traits of economic importance in racing animals (Desgorces *et al.*, 2012; Ekiz and Kocak, 2005; Ekiz *et al.*, 2005a; Ryan, 1975; Taubert *et al.*, 2007; Thiruvankadan *et al.*, 2009a,b; Villela *et al.*, 2002).

1.10.5. Genetic Evaluations and Application of EBVs to Livestock Breeding Programmes

Within the livestock industries, traditional methods of animal selection for breeding are based on visual inspection of individuals, for example for conformation traits, and on phenotypic performance in traits such as milk yield, fertility or growth rate. Recent scientific advancements and development of genetic selection technologies have enabled an animal's true genetic merit, or breeding value, to be estimated more accurately. Over the past 50 to 60 years, the calculation and incorporation of EBVs into breeding programmes, and selection of animals with superior EBVs for desirable traits, have revolutionised livestock production worldwide.

In the dairy cattle industry, genetic evaluations and EBVs are used in breeding programmes for a range of economically-important and health-related traits, including milk production traits of total yield, fat and protein percentage (Kaygisiz, 2013; Visscher *et al.*, 1991); conformation and linear type traits (Kougioumtzis *et al.*, 2014; Zink *et al.*, 2011); fertility and calving traits (Eaglen *et al.*, 2012; Pritchard *et al.*, 2013b; Royal *et al.*, 2002a, 2002b; Wall *et al.*, 2003); health traits and resistance to certain common diseases (Pritchard *et al.*, 2013a, 2013b; Stott *et al.*, 2005; Weber *et al.*, 2013); feed intake, live weight and body condition score (Buttchereit *et al.*, 2012; Pech *et al.*, 2014); and workability traits such as temperament and ease of milking (Sewalem *et al.*, 2011). Traits recorded and used in genetic evaluations and breeding programmes for beef cattle and sheep include reproductive traits (Amer *et al.*, 1998; Bormann and Wilson, 2010; de Vries *et al.*,

1998; Macfarlane *et al.*, 2010; Roughsedge *et al.*, 2005); growth and carcass traits (Amer *et al.*, 1998; Bouquet *et al.*, 2010; Mortimer *et al.*, 2014); wool quality in sheep (Safari *et al.*, 2005; Swan *et al.*, 2008); and milk production and udder conformation traits in dairy ewes (Carta *et al.*, 2009). Genetic evaluations and EBVs are also widely used for breed improvement programmes in pig, poultry and fish industries (Bourdon, 2014).

1.10.6. Genetic Evaluations and Application of EBVs to Equine Breeding Programmes

There is a long recorded history of unidirectional selection and clearly defined selection criteria within horse breeds. Selection criteria similar to those used today for selection of riding and racing horses had been defined for cavalry horses by 400 BC (Evans *et al.*, 1995). Over the past 300 years, Thoroughbred horses have been selectively bred to improve performance traits such as race time, rank finish position, stamina and earnings. Breeding programmes commonly involve traditional methods of selection based on phenotypic performance. Genetic evaluations of horse populations and application of EBVs to breed improvement programmes, however, are minimal in comparison to those undertaken for the food-producing livestock industries (Thiruvankadan *et al.*, 2009a).

Equine breeding selection has been developed by applying modern methods of genetic analysis to calculate estimated heritabilities (Table 1.3) for a number of race performance traits in Thoroughbreds (Bakhtiari and Kashan, 2009; Buxadera and Mota, 2008; Ekiz *et al.*, 2005b; Ekiz and Kocak, 2007; Mota *et al.*, 1998, 2005;

Oki *et al.*, 1995; Park, 2011; Sobczynska and Lukaszewicz, 2004; Svobodova *et al.*, 2005; Taveira *et al.*, 2004; Williamson and Beilharz, 1998); Arabian racehorses (Ekiz and Kocak, 2005; Ekiz *et al.*, 2005a); trotting racehorses (Bugislaus *et al.*, 2004; Gomez *et al.*, 2010a, 2010b, 2011; Langlois and Vrijenhoek, 2004); and Quarter horses (Correa and Mota, 2007; Villela *et al.*, 2002).

1.10.6.1. *Thoroughbred Racehorses*

Competition data are frequently used in the genetic evaluation of racehorses. The performance trait 'race time' is a direct measure of speed over a certain distance. Reported h^2 estimates for race time of Thoroughbred horses vary from 0.06 to 0.84 according to age, sex, track, race distance and h^2 estimation method (Thiruvankadan *et al.*, 2009a; Williamson and Beilharz, 1996), with a tendency for h^2 estimates to decrease as the race distance increases (Bakhtiari and Kashan, 2009; Ekiz and Kocak, 2007; Park 2011; Mota *et al.*, 2005; Mota, 2006; Oki *et al.*, 1995; Williamson and Beilharz, 1998). Similar results have been reported in the American Quarter Horse (Buttram *et al.*, 1988). This may be due to other factors, such as the jockey and temporary environmental effects, having a stronger effect on racing speed and performance as the race distance increases (Oki *et al.*, 1995), suggesting that racehorse selection based on racing time may become less effective with increasing race distance (Thiruvankadan *et al.*, 2009a). Oki *et al.* (1995) suggested that racing time at different distances might be regarded as different traits.

In contrast, Ekiz *et al.* (2005a,b) reported a marked increase in h^2 estimates for a race distance of 1,600 m, when compared to races at 1,200 m. Buxadera and Mota (2008) reported a different pattern for race performance of Thoroughbred horses in Brazil, with h^2 estimates for race time remaining relatively constant (0.22 to 0.21) for 1,000 m, 1,100 m and 1,200 m race distances, then declining with greater distances until reaching a h^2 of 0.10 at 1,600 m. Mota (2006) reported h^2 estimates for Thoroughbred horses in Brazil varying from 0.10 to 0.32 according to race distance, with the highest h^2 estimate for the shortest race distance of 1,000 m. Mota (2006) suggested that selection based on racing time at 1,000 m could be effective within a breeding program; as this trait shows higher h^2 (0.32), and genetic correlations between race time at different distances were found to be favourable and relatively high (mean of 0.74).

1.10.6.2. *Sports Horses*

In sports horses, heritabilities have been published for jumping, dressage and cross-country performance, longevity, temperament, conformation and movement analysis traits (Aldridge *et al.*, 2000; Kearsley *et al.*, 2008; Posta *et al.*, 2009, 2010; Ricard and Blouin, 2011; Stewart *et al.*, 2010, 2011; Viklund *et al.*, 2010). The use of genetic evaluations in sport horse breeding programmes is commonly performed in Sweden (Ollson *et al.*, 2008; Viklund *et al.*, 2008, 2010; Wallin *et al.*, 2003), the Netherlands (Ducro *et al.*, 2007; Huizinga *et al.*, 1991a, 1991b; Koenen *et al.*, 1995), Germany (Becker *et al.*, 2011; Bruns *et al.*, 1980), France (Dubois *et al.*, 2008; Langlois and Blouin, 2004; Ricard and Blouin, 2011;

Ricard and Chanu, 2001), Belgium (Rustin *et al.*, 2009), Hungary (Posta *et al.*, 2009, 2010) and Ireland (Aldridge *et al.*, 2000).

Data from young sport horse performance tests or adult competition data, or a combination of both, are often used in the calculation of genetic parameters. The published EBVs are routinely and successfully used in many international sport horse breeding programmes (Stewart *et al.*, 2011). Selection indexes combining breeding values for several traits of interest are now calculated routinely in many different horse breeds (Barrey, 2010). Performance tests for young sports horses were more recently introduced in the UK in 2002. Several studies have performed genetic evaluations of British sport horses, using young horse test data (Stewart *et al.*, 2011) or adult competition data (Kearsley *et al.*, 2008; Stewart *et al.*, 2010). Applications of genetic evaluations and EBVs to British breed improvement programmes are currently under development.

1.10.7. Canine Breed Improvement Programmes

1.10.7.1. *Domestication of the Dog*

Until recently, domestication of the dog was believed to have begun approximately 12,000 to 14,000 years ago. Domestic dog remains dating back more than 30,000 years, however, have recently been discovered in central Europe, indicating a much earlier time of domestication (Vila and Leonard, 2012). Several morphologically divergent dogs, including Sighthounds and Mastiffs, were

depicted on ancient Egyptian tombs, ancient Greek vases and in western Asia approximately 4,000 years ago (Vila and Leonard, 2012). The Romans were possibly the first to develop distinct breeds of dog that differed dramatically in conformation and size.

A population bottleneck is a dramatic reduction in the size of a population due to events such as environmental disasters, habitat destruction or the hunting of a species to the point of extinction. Such events can result in the loss of genetic variation within a population, as many alleles that were present in the original population are lost. Consequently, the remaining population demonstrates a low level of genetic diversity, and can eventually become genetically distinct from the original population. It is therefore hypothesised that population bottlenecks can result in the evolution of new species (Nature Education, 2014).

The population history of the dog includes two population bottlenecks; the first approximately 7,000 to 50,000 generations ago as a result of domestication from wolves, and the second approximately 50 to 100 generations ago due to intensive selection by humans creating hundreds of genetically isolated canine breeds which vary considerably in morphology, disease susceptibility and behavioural traits (Lindblad-Toh *et al.*, 2005). Most breeds were created using small numbers of founders, with selection for traits of interest such as herding, hunting and obedience abilities or desired physical characteristics. The use of popular sires, strict breeding programmes and periodic population bottlenecks during the World Wars have led to the development of closed breeding populations (Lindblad-Toh *et*

al., 2005; Parker and Ostrander, 2005). The unique breeding history and population structure of the domestic dog has therefore resulted in extreme genetic and phenotypic diversity between breeds, but limited heterogeneity within breeds and relatively little recombination since the last bottleneck (Ostrander and Ruvinsky, 2012). The domestic dog is now the most phenotypically diverse mammalian species. There are approximately 400 breeds recognised worldwide, all demonstrating dramatic differences in appearance, conformation, disease susceptibility and reported behaviour (Parker, 2012).

1.10.7.2. Pedigree Dog Breeding

In recent years, pedigree dog breeding has attracted increased media and public interest (Higgins and Nicholas, 2008). This had led to the commission of several reviews on dog breeding and investigations into the relatively high prevalence of disease amongst purebred canine breeds: The Independent Inquiry into Dog Breeding (Bateson, 2010); the APGAW inquiry into the health and welfare issues surrounding the breeding of pedigree dogs (APGAW, 2009); and an independent scientific report commissioned by the Royal Society for the Prevention of Cruelty to Animals (RSPCA) (Rooney and Sargan, 2008). The health and welfare of pedigree dog breeds can be improved through the optimisation of breeding programmes and targeted genetic selection, in order to reduce the prevalence of inherited disease (Lewis *et al.*, 2010b). Historically, breed improvement within the canine species has been largely based on phenotypic selection; a slow and inefficient

method of animal improvement (Ostrander and Ruvinsky, 2012; Taubert *et al.*, 2007).

Successful breeding programmes require comprehensive data collection systems, methods of recording information for individual animals, thorough genetic evaluation and an effective way of implementing and monitoring the breeding programme (Lewis *et al.*, 2010b). Historically, there have been very few recording systems in place for collection of population-wide canine health, welfare and performance data, and no centralised database of individual animal information and pedigree records (Lewis *et al.*, 2010b). Consequently, in comparison to the livestock industries, the majority of dog breeders have limited access to this information.

Over the past 30 years, the British Veterinary Association (BVA) and UK Kennel Club (KC) have jointly developed voluntary recording schemes for several canine diseases, including hip dysplasia, elbow dysplasia, several inherited eye diseases, Chiari malformation and syringomyelia. These recording schemes represent the main British canine breeding programmes with standardised data collection and a centralised database of information, to aid in the phenotypic selection of animals for breeding (British Veterinary Association, 2014; Kennel Club, 2014; Lewis *et al.*, 2010b).

An additional requirement for a successful breeding programme is the ability to convince breeders and owners of its importance for the positive improvement of

the breed. Furthermore, breeders must be encouraged to apply sufficient selection pressure to health, welfare and performance traits to ensure that significant progress can be made (Lewis *et al.*, 2010b). This may present a challenge in dog breeding, as traditionally pedigree dogs have been bred mainly for appearance and conformation traits, with minimal amounts of selection pressure applied to breeding for health and welfare traits (Lewis *et al.*, 2010b).

1.10.7.3. *Genetic Evaluations and Application of EBVs to Canine Breeding Programmes*

In recent years, the increasing availability of canine pedigree records and phenotypic, health and performance data has enabled canine breeders to make use of the genetic analysis and statistical tools long employed by the livestock industry (Famula, 2012). EBVs have recently been calculated for several complex inherited canine diseases, including elbow and hip dysplasia in several dog breeds, and mitral valve disease and syringomyelia in Cavalier King Charles Spaniels (Lewis *et al.*, 2010b,c, 2011). In addition, genetic evaluations have been reported for several behavioural traits including fearfulness in guide dogs (Goddard and Beilharz, 1985); hunting behaviour in Swedish Flat-coated Retrievers (Lindberg *et al.*, 2004); aggression-related traits in Golden Retrievers (Liinamo *et al.*, 2007), and seven different behavioural traits in German Shepherd Dogs (Ruefenacht *et al.*, 2002).

Canine hip dysplasia (CHD) is a developmental orthopaedic disorder affecting the coxofemoral (hip) joint, in which malformation and instability of the joint leads to

abnormal wearing of the bone surfaces and the eventual development of osteoarthritis. CHD is a complex genetic disease involving both genetic and environmental components, and is commonly observed in larger breeds such as the Labrador Retriever (Fossum, 2007).

Efforts have been made to reduce the prevalence of CHD through international recording schemes, including the voluntary BVA and KC hip scoring scheme established in 1984 (Gibbs, 1997) and currently operational in the UK, Ireland, Australia and New Zealand. The hip scoring schemes are based on phenotypic scoring of CHD status through radiographic examination and evaluation. This information is made available to breeders and dog owners, to aid in the phenotypic selection of animals for breeding.

Several studies have reported CHD to be a moderately heritable trait, with h^2 estimates ranging from 0.20 to 0.60 (Breur *et al.*, 2012; Leppanen *et al.*, 2000; Malm *et al.*, 2008) according to the type of phenotypic hip score used in the genetic analyses. Heritability ranges from 0.2 to 0.3 for hip scores based on the ventrodorsal, extended hip joint radiograph of the pelvis, and from 0.5 to 0.6 for the distraction index (measurement of hip joint laxity) and dorsolateral subluxation hip scoring method (Breur *et al.*, 2012).

There are varying reports on the success of phenotypic hip recording schemes and the international response to selection against CHD. Kaneene *et al.* (1997) and Zhang *et al.* (2009) reported a modest improvement in hip joint phenotypes of

purebred dogs in the USA. A steady genetic improvement has been reported in the Swedish Rottweiler and the Bernese Mountain Dog (Malm *et al.*, 2008), and in a retrospective study of Labrador Retrievers in the USA over a period of four decades (Hou *et al.*, 2010). A reduction in prevalence of CHD has been reported in some Finnish registered breeds, however an increased prevalence reported in others (Leppanen and Saloniemi, 1999). In the UK, a study by Willis (1997) reported that there was no improvement in hip joint phenotypes of five breeds between 1987 and 1995. A recent study of Labrador Retrievers in the UK found a consistent but slow genetic improvement in hip scores between 2000 and 2007, approximately equivalent to avoiding breeding from dogs with the worst 15% of scores (Lewis *et al.*, 2010b).

Overall, the rate of improvement in such joint disorders has been slower than desirable. The BVA and KC are now moving from phenotypic selection towards the use of pedigree information and the selection of animals for breeding based on EBVs (Lewis *et al.*, 2010b). Many authors recommend that canine hip and elbow dysplasia control schemes could be improved through EBV-based selection (Ginja *et al.*, 2008, 2010; Hou *et al.*, 2010; Leppanen and Saloniemi, 1999; Lewis *et al.*, 2010b, 2010c, 2013; Malm *et al.*, 2008; Smith *et al.*, 2001; Wooliams *et al.*, 2011; Zhang *et al.*, 2009; Zhu *et al.*, 2009). This method would increase the effectiveness of selection, and result in a faster rate of genetic improvement compared to breeding based on phenotypic selection alone. In a study of CHD in British Labrador Retrievers, deterministic analysis supported by simulations found that the use of EBVs, rather than phenotypic selection, could increase the rate of

genetic progress by 19% through increasing accuracy of selection, even if the selection intensity remains unchanged (Lewis *et al.*, 2010b).

EBV-based selection against hip and elbow dysplasia has been introduced in several countries including Germany, Finland, Norway, Denmark, Sweden, the USA, and more recently the UK (Kennel Club, 2014; Lewis *et al.*, 2013; Malm *et al.*, 2008). In the USA, the Cornell University College of Veterinary Medicine has developed a public online database of EBVs for Labrador Retrievers, derived from hip scores and pedigrees (Hou *et al.*, 2010; Ostrander and Ruvinsky, 2012). This data is available to assist breeders in the selection of Labrador Retrievers based on their genetic potential for better hip quality. In the UK, EBVs for hip and elbow dysplasia in the Labrador have recently been published (Lewis *et al.*, 2013) through a joint scheme with the British Animal Health Trust (AHT) and KC. The EBVs are made publically available for all KC-registered Labradors through the online 'Mate Select' tool (Kennel Club, 2014).

In addition, several service dog organisations are performing EBV-based selection for breeding. Tricuspid valve dysplasia is a congenital malformation of the tricuspid valve complex of the cardiovascular system, resulting in valvular insufficiency and systolic regurgitation of blood into the right atrium of the heart (Merck Veterinary Manual, 2016). Guide Dogs for the Blind in San Rafael, California, have developed selection indices in order to improve several behavioural and health-related traits, such as tricuspid valve dysplasia in Labrador Retrievers. Since the introduction of EBV-based selection against tricuspid valve dysplasia in 2008, there has been a

steady decline in the genetic risk of the condition in this population of service dogs (Famula, 2012). Within the Guiding Dogs for the Blind organisation in the USA, the application of hip EBVs to the selection process for breeding has also resulted in genetic improvement in hip conformation (Zhang *et al.*, 2009).

1.10.8. Canine Genomics

The domestic dog has a total of 78 chromosomes; 38 pairs of autosomes and one pair of sex chromosomes (Ostrander and Ruvinsky, 2012).

1.10.8.1. Sequencing of the Canine Genome

The international Dog Genome Project was led by researchers at the Broad Institute of MIT and Harvard. As a result, the canine genome was sequenced in 2005 from a female Boxer dog (Lindblad-Toh *et al.*, 2005), just two years after completion of the Human Genome Project. The current high-quality canine genome has 7.5 times genome coverage and includes approximately 99% of the 2.4 billion base pair (bp) euchromatic genome of a female Boxer dog (Karlsson and Lindblad-Toh, 2008; Lindblad-Toh *et al.*, 2005). The euchromatic section of the canine genome is approximately 450 million bp (19%) smaller than the human genome (Karlsson and Lindblad-Toh, 2008; Lindblad-Toh, 2012). The assembly of the dog genome (CanFam1.0 and CanFam2.0) was deposited in public databases, and a new annotation (CanFam3.1) was produced in September 2011 by the Broad Institute of MIT and Harvard.

A Single Nucleotide Polymorphism (SNP) is a variation at a single nucleotide position [Adenine (A), Thymine (T), Cytosine (C) or Guanine (G)] in a DNA sequence between individual members of a species (Figure 1.7).

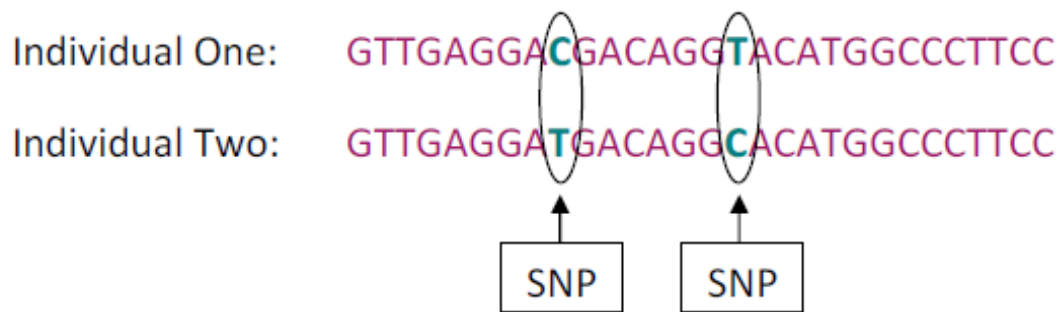


Figure 1.7. Example of a Single Nucleotide Polymorphism (SNP) at two different nucleotide positions in a DNA sequence from two individuals.

SNPs occur throughout the entire genome, either within coding sequences of genes, non-coding regions or intergenic regions. SNPs within coding regions can be classified as synonymous, which do not alter the encoded amino acid, or as nonsynonymous, which change the amino acid incorporated into the polypeptide (Nicholas, 2003).

An extensive canine SNP map, containing 2.5 million SNPs, was developed by comparing the Boxer dog genome (CanFam1.0 and CanFam2.0) with the partial sequence of a Standard Poodle (1.5 times genome coverage) generated previously (Kirkness *et al.*, 2003), and with approximately 100,000 sequence reads from each

of nine dog breeds, and approximately 22,000 sequence reads from each of four grey wolves and one coyote (Lindblad-Toh *et al.*, 2005). This high-density SNP map exhibits even distribution across the canine genome and high between-breed polymorphism, and can therefore be used for genetic studies in any breed of dog.

Several canine whole-genome SNP genotyping arrays have been produced and are available for genetic studies, with increasing numbers and quality of SNPs on the most recent releases. First-generation arrays consisted of the 22,362 SNP Illumina array (Illumina, San Diego, CA), the 26,578 SNP Affymetrix Canine GeneChip and the 49,663 SNP Affymetrix Canine GeneChip (Affymetrix, Santa Clara, CA). Two second-generation arrays are currently available: the 170,000 SNP Illumina CanineHD BeadChip (Illumina, San Diego, CA) and the 127,000 SNP Affymetrix Canine Genotyping Array (Affymetrix, Santa Clara, CA).

1.10.9. Approaches to the Genomic Study of Complex Diseases

1.10.9.1. *Candidate Gene Association Studies*

Family-based linkage analysis studies have been successful in mapping genetically homogenous rare Mendelian disorders, but have been unsuccessful for mapping heterogenous complex human traits (Altmuler *et al.*, 2001). Population-based genetic association studies were first carried out in the late 1990s, based on the idea that common genetic variants may underlie many common traits or diseases (Zondervan, 2011). Candidate gene association studies investigate associations

between the genetic variation occurring within a pre-determined specific gene or locus of interest and a particular phenotype, using case-control designs to identify polymorphisms, such as SNPs, within candidate genes (Teare, 2011). The candidate genes are usually selected for study based on prior knowledge or biological hypotheses of a gene's involvement in particular biological pathways likely to be involved in the phenotype. This type of study is more suitable than linkage mapping for detecting genes underlying more common and complex diseases, where the risk associated with any given gene is relatively small. Candidate gene studies are often biased, however, and limited to small regions of genes that have been previously linked with the phenotype.

1.10.9.2. Genome-Wide Association Studies (GWAS)

Genome-Wide Association Studies (GWAS) use an unbiased hypothesis-free approach to investigate genetic variations associated with a particular phenotype, by examining known SNPs across the entire genome in a case-control study design. Genotypes of affected (case) and unaffected (control) individuals are compared, in order to detect differences in the DNA sequence and therefore identify regions of chromosomes that may be associated with the trait of interest. GWAS are particularly useful for detecting genetic variations that contribute to common, complex diseases, and have become the method of choice for such studies (Iles, 2011).

In humans, GWAS have been shown to be an effective and comprehensive approach for identifying genetic variations associated with many complex diseases (Wellcome Trust Case Control Consortium, 2007). The sequencing of the canine genome, development of an extensive SNP map and production of whole-genome SNP genotyping arrays have recently enabled the use of GWAS to investigate polymorphisms associated with disease in many breeds of dog (Baird *et al.*, 2014; French *et al.*, 2012; Massey *et al.*, 2014; Tsai *et al.*, 2012; Wood *et al.*, 2009; Zhao *et al.*, 2013).

1.11. Greyhound Breeding

1.11.1. The Greyhound Breeding Industry

First published in 1882, the British Greyhound Stud Book is the official registry of Greyhounds bred in the UK, and also registers litters, matings, stud dogs, breeders, owners and syndicates (Greyhound Stud Book and National Coursing Club, 2014). Unlike many other canine breeds, the Greyhound has been relatively unaffected by artificial selection based on appearance, as selection for breeding is still mainly based on race performance and speed (Davis, 1967).

Large numbers of Greyhounds are bred to supply the British racing industry. Given the relatively short racing career of a Greyhound of approximately two to four years, there is a high turnover of dogs. An estimated 10,000 to 11,000 Greyhounds

enter and leave the licensed sector each year, with an additional 3,000 to 4,000 racing on Independent tracks (Donoughue, 2007). The reports of the APGAW inquiry into the Welfare of Racing Greyhounds in the UK (APGAW, 2007) and Independent review commissioned by the British Greyhound Industry (Donoughue, 2007) both highlighted that the Greyhound Industry produces a surplus of unwanted young dogs that do not make the grade to race competitively.

Racing Greyhounds are currently produced by both commercial suppliers and through smaller-scale breeding by owners and trainers. Ireland has a very successful Greyhound industry and breeds over 20,000 Greyhounds per year; a large number of which are used to supply the British racing industry (APGAW, 2007; Donoughue, 2007). In 2006, there were 608 Greyhound litters registered with the British Greyhound Stud Book, compared to 4,481 litters registered in Ireland with the Irish Greyhound Stud Book (APGAW, 2007). As the average litter size for Greyhounds is between six and seven puppies (Genders, 1975; Lennox, 1987), this suggests that in 2006, approximately 4,256 Greyhounds were bred in the UK and 31,367 were bred in Ireland (APGAW, 2007). During recent years, over 8,000 Greyhounds were imported annually from Ireland to the UK (Donoughue, 2007). Overall, approximately 75% of Greyhounds that race in the UK were originally bred in Ireland, with the remaining 25% bred in Britain (APGAW, 2007).

Commercial dog breeders in the UK are licensed and inspected under the Breeding and Sale of Dogs (Welfare) Act 1999. Currently, however, there is no comprehensive monitoring or licensed regulation of Greyhound breeding, no

regulatory inspection of breeding kennels, and little enforcement of the Breeding and Sale of Dogs (Welfare) Act 1999 by the British Greyhound industry (Donoughue, 2007). Since the early 1990s, the use of artificial insemination (AI) to produce racing Greyhounds has become more prevalent worldwide. By 2003, 86% of the National Greyhound Association-registered dogs born in the USA were produced using AI (Turner, 2010). Greyhound semen can be collected, stored and transported for AI worldwide, therefore removing the requirement for the sire and dam to be present at the same location. Furthermore, long-term storage of frozen semen enables a Greyhound sire to continue to produce offspring long after their death. In comparison to natural mating, the use of AI considerably increases the potential genetic contribution of an individual sire to the racing Greyhound population.

Popular Greyhound sires are often used intensively for breeding. 'Top Honcho', for example, was born in Australia in 1993 and is one of the most highly regarded Greyhound sires worldwide, producing a total of 10,462 offspring to date (Greyhound-Data, 2016). In a study of 42,880 Irish Greyhounds over a four-year period from 2000-2003, Taubert *et al.* (2007) found that 43.7% of the total number of offspring in the study population had descended from just 18 Greyhound sires used extensively for breeding, each with over 500 progeny. The total number of active Greyhound sires within the racing industry is, to date: 272 in Ireland, 123 in the UK, 380 in Australia and New Zealand, 135 in the USA, and 158 in other countries worldwide (Greyhound-Data, 2016).

For centuries, Greyhounds have been subjected to intense selective breeding in an attempt to produce faster and better-performing dogs (Denny, 2008). Over the past 50 to 60 years, modern methods of genetic evaluation have transformed breed improvement programmes for many domestic species. The selection of Greyhounds for breeding, however, is currently based on their own or their relative's phenotypic (observed) racing performance; a slow and inefficient method of animal improvement (Taubert *et al.*, 2007). This traditional approach to breeding usually involves selection of dogs, or relatives of dogs, that have performed well in terms of racing speed or number of races won. The most highly regarded Greyhound sires and dams have commonly achieved success at high profile race events involving large amounts of prize money (Amphlett, 1993; Clarke, 1980; Genders, 1975). The genetic merit of the Greyhound sire or dam, however, is rarely considered.

Furthermore, it is common practice in the Greyhound industry to breed from dogs that have been retired at a young age due to serious injury and are no longer able to race, therefore maintaining the dog's economic purpose (Genders, 1975). In this case, the selection of the dog for breeding is largely based on circumstances rather than targeted selection based on genetic merit. In addition, the frequent use of retired injured Greyhounds for breeding purposes within the industry may actually be selecting for weaker and more injury-susceptible dogs in the next generation.

1.11.2. Genetic Analysis of Racing Performance in the Greyhound

Greyhound running speed, or race time as a direct measure of speed, is a complex quantitative performance trait affected by both genetic and non-genetic (environmental) factors. In contrast to other animal groups, there are only three reported genetic studies investigating racing performance of the Greyhound (Desgorces *et al.*, 2012; Ryan, 1975; Taubert *et al.*, 2007). Two of these studies analysed the performance of Greyhounds racing in Ireland. Importantly, there have been no previous genetic studies investigating the race performance of British Greyhounds.

Ryan (1975) investigated the inheritance of track performance in Irish Greyhounds and calculated genetic parameters for the trait 'Racing Time'. Greyhound EBVs, however, were not calculated. A later study by Taubert *et al.* (2007) investigated genetic and environmental variation in race performance data of 42,880 Irish Greyhounds, racing over a distance of 480 m. The data consisted of 239,829 performance results for 42,785 flat races at 20 different race tracks in Ireland, over a four-year period from 2000 to 2003. The performance traits 'Racing Time' (the time in seconds for a Greyhound to complete a race over a distance of 480 m), 'Adjusted Racing Time' (a scaled logarithmic function for Racing Time, used to adjust Racing Time to be normally distributed) and 'Ranking' (the finish position of a Greyhound in a race) were investigated. Taubert *et al.* (2007) reported moderate heritabilities of 0.31 and 0.38 for the performance traits Racing Time and Adjusted

Racing Time, respectively, and a low h^2 of 0.10 for Ranking. Meissen (1997) reported similar results in a study of racing performance of Whippets, where as Ryan (1975) reported a slightly lower h^2 of 0.23 for Racing Time in Irish Greyhounds over a 480 m race distance. In a further genetic study of racing Greyhounds, Desgorces *et al.* (2012) compared the running speeds of the 10 best performing Greyhounds worldwide (2007-2009) with the individual best performance of their male ancestors over seven generations, and reported a low h^2 of 0.18 ($p = 0.08$) for the trait 'Running Speed' (Table 1.3).

Taubert *et al.* (2007) calculated EBVs for each Greyhound in their data set, and reported EBVs ranging from 0.62 to -0.78 (SD 0.15) for the performance trait Racing Time, and from 1.05 to -1.30 (SD 0.25) for the performance trait Ranking. Interestingly, for the trait Racing Time, there was considerable variation in EBVs (0.45 to -0.01) between the 10 Greyhound sires with the highest number of offspring (851 to 2,031 progeny). Consequently, the 10 Greyhound sires used most intensively for breeding in Ireland were not the dogs with the best EBVs, and only one of the 10 dogs with the highest number of offspring had an EBV among the top 30 evaluated Greyhounds. The dog with the eighth highest number of offspring (991 progeny) actually had a negative (less favourable) EBV of -0.01.

Taubert *et al.* (2007) found a positive phenotypic and genetic trend in Racing Time of Irish Greyhounds over the years 2000 to 2003, with a phenotypic improvement of approximately 0.125 seconds per year and a genetic improvement of approximately 0.03 seconds per year. While this indicates that the current system

of selection for breeding based on phenotypic race performance is effective, as Racing Time is a moderately heritable trait, the study demonstrates that the genetic potential of the Irish Greyhound population is not being used at a maximum. Importantly, the authors conclude that EBV-based selection of racing Greyhounds for breeding would be considerably more efficient.

The targeted genetic selection of racing Greyhounds would therefore result in a greater and faster rate of genetic improvement within the population, compared to breeding based on phenotypic selection alone. Furthermore, selection based on EBVs would considerably increase the efficiency of racing Greyhound production. This would potentially bring economic benefits to the entire Greyhound industry, including breeders, owners, trainers, and rehoming and rescue organisations. Importantly, the use of targeted genetic selection based on EBVs may improve Greyhound welfare by enabling breeders to produce Greyhounds of predictable and superior genetic merit, therefore considerably reducing the over-breeding and wastage of young dogs in the industry. A reduction in the quantity of 'unwanted' dogs produced would subsequently reduce numbers requiring rehoming, and would therefore decrease the current strain on rehoming charities and rescue organisations. As a result, this may reduce the potentially large numbers of young Greyhounds that are euthanased each year simply because they do not make the grade to race.

1.12. Measures of Stress in Canine Athletes

1.12.1. Physiological Effects of Intense Exercise in the Racing Greyhound

Greyhounds exhibit unique physiological characteristics and adaptations as racing sighthounds, including higher resting haematocrit, haemoglobin concentration and red blood cell counts, and lower leukocyte and platelet counts, compared to other canine breeds (Campora *et al.*, 2011; Zaldivar-Lopez *et al.*, 2011).

Haematology and biochemistry parameters can be used to assess health and fitness in canine athletes, and to monitor the physical stress that occurs during exercise (Rovira *et al.*, 2007). Racing Greyhounds typically take part in short-duration intense exercise activity, competing over race distances of 235 m to 800 m, with maximum speeds of 18 m/s (Dobson *et al.*, 1988). Such activity has been found to cause extreme changes in many physiological variables in racing Greyhounds, including haematologic, biochemical, blood gas and acid base values. This often results in fluid, electrolyte and packed cell volume shifts and mild hyperglycaemia post-racing (Dobson *et al.*, 1988; Ilkiw *et al.*, 1989; Nold *et al.*, 1991; Rose and Bloomberg, 1989; Snow *et al.*, 1988; Toll *et al.*, 1995). Sprinting has been associated with muscle damage in Greyhounds, with increases in plasma muscle enzyme activities (creatine kinase [CK], aspartate aminotransferase [AST] and lactate dehydrogenase [LDH]) post-exercise, that persist for three hours after racing (Ilkiw *et al.*, 1989). Plasma and whole blood haematocrit, arterial pH and

concentrations of potassium [K^+], total protein and lactate, were found to change significantly with increasing race length and exercise duration (Nold *et al.*, 1991).

1.12.2. Cortisol as a Measure of Stress

Environmental stimuli that result in an imbalance of homeostasis are often referred to as 'stressors', and the subsequent physiological reaction of the animal as a 'stress response' (Mostl and Palme, 2002). Various endocrine activities, in particular the release of glucocorticoid and catecholamine hormones, are involved in the stress response. Cortisol is a glucocorticoid hormone secreted by the adrenal gland due to activation of the hypothalamic-pituitary-adrenal (HPA) axis, in response to external and internal stimuli (Matteri *et al.*, 2000; Mostl and Palme, 2002). The production, metabolism and excretion of cortisol is summarised in Figure 1.8.

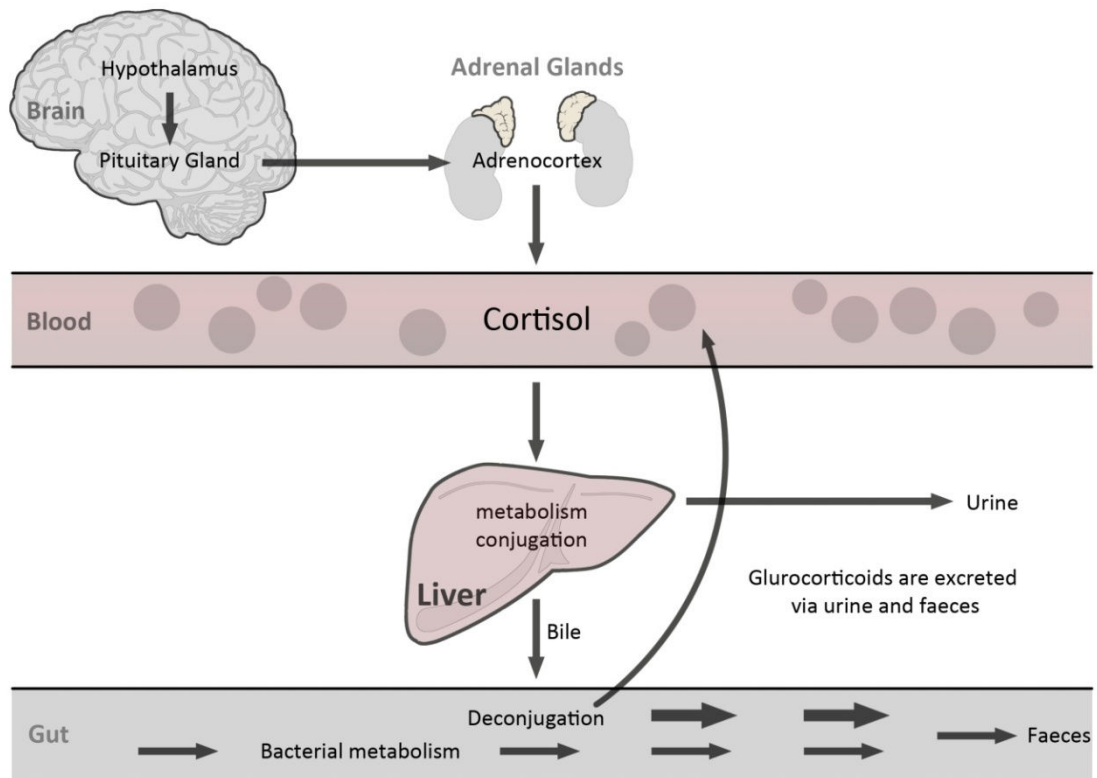


Figure 1.8. Diagram of the secretion, metabolism and excretion of cortisol. Adapted from Mostl and Palme (2002).

Glucocorticoids act on various target tissues and organs to maintain homeostasis, and are involved in gluconeogenesis by stimulating the liver to convert fat and protein to glucose for energy. Glucocorticoids also potentiate the synthesis of epinephrine (adrenaline), a catecholamine hormone secreted by the adrenal gland during the stress response. Adrenaline stimulates gluconeogenesis and lipolysis, enabling mobilisation of stores for the 'flight or fight' response. An increase in glucocorticoids inhibits further HPA response to stress through a negative feedback mechanism, in order to maintain homeostasis (Matteri *et al.*, 2000).

Acute endocrine responses to stress have important adaptive functions and are essential for coping and survival (Matteri *et al.*, 2000). The term 'stress' often implies a negative experience. As a physiological mechanism, however, stress in animals may be considered as positive, for example due to excitement and increased levels of arousal resulting in adaptive responses such as increased alertness and preparation for activity (Pastore *et al.*, 2011). During short-term stress, secretion of glucocorticoids improves fitness through energy mobilisation (Raynaert *et al.*, 1976). Glucocorticoids are also released during animal courtship, copulation and hunting (Broom and Johnson, 1993). Long-term stressors and chronic elevation of glucocorticoids, however, may result in protein catabolism, hyperglycaemia, immunosuppression and reduced reproductive performance (Dobson and Smith, 1995; Matteri *et al.*, 2000; Mostl and Palme, 2002).

Cortisol is a frequently used measure of physiological stress in most mammals, including humans (Stawski *et al.*, 2013), dogs (Beerda *et al.*, 1996, 1998; Hekman *et al.*, 2012; Kobelt *et al.*, 2003; Pastore *et al.*, 2011), horses (Fazio *et al.*, 2008a,b, 2013; Schmidt *et al.*, 2010a,b,c,d), sheep (Hargreaves and Hutson, 1990), cattle and pigs (Von Borell *et al.*, 2007). Cortisol is commonly used as a marker for assessment of canine welfare (Menor-Campos *et al.*, 2011), reaction to stress challenges (Bergeron *et al.*, 2002) and human-animal interaction studies (Bergamasco *et al.*, 2010; Jones and Josephs, 2006), and has been shown to be a useful measure of acute and chronic physiological and psychological stress in dogs. The use of cortisol as a measure of stress in racing Greyhounds, however, has not previously been reported.

1.12.3. Additional Measures of Stress

The autonomic nervous system functions to maintain homeostasis through its control of visceral organs and glandular secretions. There are two branches of the autonomic nervous system, the sympathetic and parasympathetic, which work together (usually antagonistically) to maintain homeostasis (Uemura, 2015). During an animal's 'fight or flight' response (Cannon, 1929) to stressful stimuli, stimulation of the sympathetic division results in increased heart rate (HR), blood pressure, body temperature and gastrointestinal activity (Kay and Hall, 2009; Moberg, 2000; Uemura, 2015). HR and HR variability (short-term fluctuations in HR) are commonly used as measures of stress in horses (Becker-Birck *et al.*, 2013; Kay and Hall, 2009; Lewinski *et al.*, 2013; Schmidt *et al.*, 2010a,b,c,d) and dogs (Bergeron *et al.*, 2002), but have not previously been reported in the racing Greyhound.

Additional measures of stress include monitoring of immunological changes and susceptibility to disease (Moberg, 2000), as well as an animal's behavioural response to a stressor (Bergeron *et al.*, 2002; Hekman *et al.*, 2012; Kay and Hall, 2009; Menor-Campos *et al.*, 2011; Ottenheimer Carrier *et al.*, 2013; Pastore *et al.*, 2011).

1.13 Summary

In summary, there is an urgent need within the Greyhound industry to reduce the overproduction and wastage of young dogs that do not make the grade to race. The use of scientific methods of genetic analysis, calculation of EBVs and their application to animal breeding programmes have been very successful in improving health, welfare and performance traits in livestock species, sports horses and guide dogs (Bourdon, 2014; Famula, 2012; Simm, 1998). In contrast to other animal groups, there are only three reported genetic studies investigating racing performance of the Greyhound (Desgorces *et al.*, 2012; Ryan, 1975; Taubert *et al.*, 2007), and there have been no previous genetic studies of British Greyhounds. This thesis presents the first investigation of environmental and genetic variation in the racing performance of British Greyhounds, estimation of heritabilities for several economically-important performance traits and calculation of EBVs for individual dogs. In the only previous study to calculate EBVs for racing Greyhounds, Taubert *et al.* (2007) examined 239,829 performance records of 42,880 Irish Greyhounds over a four-year period from 2000 to 2003, and two generations of pedigree data for each dog. In the largest genetic study of racing Greyhounds worldwide, this thesis examines 1,711,489 performance records of 50,452 British Greyhounds over a longer five-year period from 2008 to 2012, and six generations of pedigree data for each dog. EBVs are calculated for the performance traits race time, speed and rank finish position, enabling individuals to be ranked in order of their additive genetic merit.

In addition, Greyhounds are prone to specific musculoskeletal injuries during their racing career, and stress fractures of the distal limb are common (Gannon, 1972; Prole, 1976; Sicard *et al.*, 1999). Stress fracture is thought to be a multifactorial multigenic disorder resulting from a combination of environmental and genetic components (Yanovich *et al.*, 2012). A number of risk factors have been identified in the development of stress fractures in human athletes and military recruits (Chatzipapas *et al.*, 2009; Friedl *et al.*, 1992; Giladi *et al.*, 1986; Givon *et al.*, 2000; Jones *et al.*, 2002; McClung and Karl, 2010; Milgrom *et al.*, 1985; Singer *et al.*, 1990). Investigation of genetic factors involved in the pathogenesis of stress fracture in the racing Greyhound, however, has not previously been reported. Following sequencing of the canine genome and the production of canine whole-genome SNP genotyping arrays, GWA studies have been used to investigate polymorphisms associated with disease in many breeds of dog (Baird *et al.*, 2014; French *et al.*, 2012; Massey *et al.*, 2014; Tsai *et al.*, 2012; Wood *et al.*, 2009; Zhao *et al.*, 2013). This thesis presents the first investigation of genetic factors that may predispose racing Greyhounds to developing stress fracture injuries, by performing a case-control GWA study to identify potentially associated SNPs.

The final study in this thesis investigates pre- and post-race salivary cortisol concentration and HR as measures of stress in British Greyhounds. Cortisol is a glucocorticoid hormone secreted due to activation of the HPA axis in response to external and internal stimuli (Matteri *et al.*, 2000; Mostl and Palme, 2002). Cortisol

is frequently used as a measure of canine stress (Beerda *et al.*, 1996, 1998; Hekman *et al.*, 2012; Kobelt *et al.*, 2003; Pastore *et al.*, 2011), yet has not previously been examined in the racing Greyhound.

In a multifactorial approach using a combination of quantitative and molecular genetics techniques, in addition to physiological measures of stress, this thesis investigates environmental and genetic factors associated with a number of complex traits important to Greyhound welfare.

1.14 Aims of the Thesis

The aims of the thesis were:

- To create a race performance and pedigree database for GBGB-licensed Greyhounds in the UK, in order to investigate the effects of genetic and environmental factors on the variation of race performance in British Greyhounds.
- To calculate variance components, estimated heritabilities and repeatabilities for a number of important performance traits within the Greyhound industry.
- To calculate EBVs for British Greyhounds for a number of industry-important performance traits, enabling individuals to be ranked in order of their additive genetic merit.
- To establish a DNA archive for British racing Greyhounds for use in future genetic research.
- To perform a discovery case-control GWA-study using SNP genotyping to investigate genetic factors involved in the pathogenesis of stress fracture injuries of the Greyhound distal limb.
- To investigate salivary cortisol and heart rate indicators of stress in the racing Greyhound, pre- and post-race.

Chapter Two.

General Materials and Methods

2. General Materials and Methods

2.1. British Racing Greyhound Pedigree and Performance Database

2.1.1. Data Collection and Creation of a Pedigree and Performance Database for British Racing Greyhounds

The GBGB publishes all race results for licensed Greyhound races in the UK on their website (Greyhound Board of Great Britain, 2009). Pedigree information, however, is limited to the sire and dam of the Greyhound only. Greyhound-Data (2014) is an international online network of Greyhound owners, trainers and breeders, publishing all available data on race results and statistics for Greyhounds and race tracks worldwide. Information held on the Greyhound-Data website is updated on a daily basis. Comprehensive maternal and paternal pedigree data for the past 400 years are published for each Greyhound, in addition to breeding information and statistics.

The GBGB declined a request to provide data sets for use in the present study, stating that their Greyhound form database is managed by an external company. The GBGB did, however, provide the author with a list of all licensed racing Greyhounds currently registered with the GBGB, to enable the manual collection of data. Information published on the GBGB website (2014) is available for non-commercial use.

In addition, written requests to the online resource Greyhound-Data to provide data sets for use in the present study received no response. Consequently, all of the raw data was collected manually using the publicly available information produced by Greyhound-Data (2014). At the time of data collection, this data was freely available for non-commercial use.

Microsoft Office Excel (Microsoft Corporation, 2007b), with custom software written using standard Visual Basic for Applications (G. J. Dockerty, Structural Response Limited, Manchester, 2012), was used to capture and record web page information on Greyhound-Data (2014). This data consisted of phenotypic, pedigree and race performance raw data for each individual Greyhound competing in all races that took place at GBGB-licensed Greyhound tracks in the UK, during a five-year period from 1st January 2008 to 31st December 2012. The software is highly customised and requires frequent modification to accommodate changing online formats. It is therefore unavailable for external use.

The Greyhound-Data website (2014) records details of race event information for all GBGB track events held in the UK. A filter was applied to display race event data over the specified time period of 1st January 2008 to 31st December 2012. An example of race event information displayed on Greyhound-Data for the year 2008 is displayed in Figure 2.1.

UserForm4

Home | Dog-Search | Tattoo | Races | Race Cards | Coursing | Tracks | Statistic | Testmating | [SHOP-UK / IE / AU / DE](#)

Login | Private Messages | [add_race](#) | [add_coursing](#) | [add_dog](#) | Membership | Ask the Vet | Newsletter | Help

Active-Sires | Sire-Pages | Classifieds | Kennels | Photos | Videos | Library | Adoption | Forum | Links | About_us | Site Usage

6086 races with country= UK, date = 2008 and any class
page <= 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61

Name of Race	Stadium	Date v	Ht	Grade	Dist m/y	Winner	Wintime film
Crayford 01 JAN 2008 HT A8	Crayford	1 JAN 2008	A8	GR S3	540 / 591	Newby Splash	34.32
Crayford 01 JAN 2008 HT A9	Crayford	1 JAN 2008	A9	GR A3	380 / 416	Vieri Intuitive	23.69
Crayford 01 JAN 2008 HT A10	Crayford	1 JAN 2008	A10	GR H2	380 / 416	Cill Dubh Wonder	24.38
HAPPY NEW YEAR FROM CORAL	Hove & Brighton	1 JAN 2008	1	GR A3	515 / 563	Dumbarton Mick	30.34
Hove & Brighton 01 JAN 2008 HT 2	Hove & Brighton	1 JAN 2008	2	GR A9	515 / 563	Killeacle Tom	31.07
Hove & Brighton 01 JAN 2008 HT 3	Hove & Brighton	1 JAN 2008	3	GR D1	285 / 312	Raithby Toure	16.54
Hove & Brighton 01 JAN 2008 HT 4	Hove & Brighton	1 JAN 2008	4	GR A11	515 / 563	Ardross Gem	31.22
Hove & Brighton 01 JAN 2008 HT 5	Hove & Brighton	1 JAN 2008	5	GR A5	515 / 563	Wintons Gayle	30.48
Hove & Brighton 01 JAN 2008 HT 6	Hove & Brighton	1 JAN 2008	6	GR A5	515 / 563	Humming Heiress	30.72
SAM KNIGHT IS 18 TODAY	Hove & Brighton	1 JAN 2008	7	GR A10	515 / 563	Adams Image	31.12
Hove & Brighton 01 JAN 2008 HT 8	Hove & Brighton	1 JAN 2008	8	GR A2	515 / 563	Venice Champ	30.21
Hove & Brighton 01 JAN 2008 HT 9	Hove & Brighton	1 JAN 2008	9	GR A1	515 / 563	Fortuna Garcia	30.13
Hove & Brighton 01 JAN 2008 HT 10	Hove & Brighton	1 JAN 2008	10	GR A4	515 / 563	Kilkenny Faith	30.68
Hove & Brighton 01 JAN 2008 HT 11	Hove & Brighton	1 JAN 2008	11	GR A8	515 / 563	Artful Diva	31.14
Hove & Brighton 01 JAN 2008 HT 12	Hove & Brighton	1 JAN 2008	12	GR A6	515 / 563	Climates Blues	30.85
www.betfred.com Stakes	Nottingham	1 JAN 2008	A1	GR A3	500 / 547	Shins Rocket	31.11
Nottingham 01 JAN 2008 HT A2	Nottingham	1 JAN 2008	A2	GR A4	500 / 547	Tidy Class Room	31.28
Nottingham 01 JAN 2008 HT A3	Nottingham	1 JAN 2008	A3	GR A2	500 / 547	Urla Club	30.62


Race Year: Year Month
 Race Date Criteria: Process if Last Race Date Occurred Within Days
 Dog: Web Date of Birth: Db Date of Birth: Records Added = Total Records Added =

Figure 2.1. An example of UK Greyhound race event information displayed on the Greyhound-Data website for the year 2008 (Greyhound-Data, 2014).

The custom software was used to capture the race event information on Greyhound-Data (2014), display this data on Excel spreadsheets and record it locally to disk as a hypertext mark-up language (html) file. Event files were stored in folders categorised by year and month. A total of 3,230 race event files were recorded, with each event file consisting of approximately 100 race event records.

The contents of each race event html file generated were then interrogated by the custom software in order to display Greyhound-Data (2014) website information about a particular race, as displayed in Figure 2.2.

UserForm4



[Home](#) | [Dog-Search](#) | [Tattoo](#) | [Races](#) | [Race Cards](#) | [Coursing](#) | [Tracks](#) | [Statistic](#) | [Testmating](#) | [SHOP-UK](#) | [IE](#) | [AU](#) | [DE](#)

[Login](#) | [Private Messages](#) | [add_race](#) | [add_coursing](#) | [add_dog](#) | [edit_this_race](#) | [Help](#)

[Active-Sires](#) | [Sire-Pages](#) | [Classifieds](#) | [Kennels](#) | [Photos](#) | [Videos](#) | [Library](#) | [Adoption](#) | [Forum](#) | [Links](#) | [About_us](#) | [Site Usage](#)

country

full meeting

date

distance

winner

trackrecord

fastest of year

last q best

this q best

q avg wintime

comment

stadium

(Trackrecord + 1st Best)

race

type

second

Lunar Vacation

Abbeyside Bart

Lunar Vacation

Boherbradda Mac

avg time

race name

Belle Vue Dash

going

grade

third

add video

add picture

1 NOV 2007

23 OCT 2008

1 NOV 2007

31 JAN 2008

this q 45 dogs

fin	name	sex	dob	color	sire	dam	time	dist	stime	box	posts	sp	kg	comment
1st	Boherbradda Mac	m	JUN 2005	BK	Hondo Black *	Boherbradda Kay	15.07			4	11	7/1		QAw, W2, ALed, (TkRec)
2nd	Mahers Boy	m	DEC 2003	BKW	Tucks Mein	Rons Luck	15.27	2 1/2		2		7/4		Crowded1
3rd	Sherling Major	m	SEP 2005	BK	Knockeevan Major	Dream On Mary	15.61	4 1/4		5	43	16/1		Crowded1
4th	Jetharts Here	m	NOV 2004	BK	Droopys Vieri	Jackies Lady	15.73	1 1/2		1		4/5F		Crowded1, Wide2
5th	Early Night	m	APR 2005	BK	Droopys Kewell	Glasha Belle	15.75	HD		3		10/1		Crd1, FcdWide2
6th	Bearside	m	APR 2005	WBD	Brookside Bear *	Marinas Tina	16.05	3 3/4		6		6/1		MsdBrk, HitRlsBadly1

Race Year

Year

2008

Month

01

Update

Race Date Criteria

Process if Last Race Date Occurred Within

☐ Days

Exit

Dog:

Web Date of Birth:

Db Date of Birth :

Records Added =

Total Records Added =


Figure 2.2. An example of information displayed on the Greyhound-Data (2014) website for a particular Greyhound race event in the UK.

A total of 318,192 race result files were generated and recorded locally on disk as html files, and were stored categorised by year, month and stadium. The custom software then populated a Microsoft Access 2007 (Microsoft Corporation, 2007a) database with the raw data. In total, 1,882,658 race performance records for 52,826 individual Greyhounds were obtained.

Similarly, the pedigree information published by Greyhound-Data (2014) was interrogated to determine the ancestry of each dog. The custom software was used to interrogate the race result files and extract the unique identification number (*GHDataID*) allocated by Greyhound-Data for each dog. This identification number was used to display the Greyhound-Data (2014) website phenotypic and pedigree information recorded for the particular dog, as displayed in Figure 2.3. The custom software then captured the information and populated the Microsoft Access 2007 (Microsoft Corporation, 2007a) database with the data.

Six generations of maternal and paternal pedigree data were collected from the Greyhound-Data resource, for each of the 52,826 individual Greyhounds in the data set. Pedigree data consisted of sire and dam racing names and years of birth, providing an overall data set of 73,346 racing Greyhounds with known familial relationships between individuals. The structure of a six-generation pedigree is displayed in Figure 2.4.

Check Web Data - Ruth Dockerty



Boherbradda Mac

color Black
sex male (active sire)
weight 34 kg ~ 75 lbs
date of birth 15 JUN 2005
land of birth IU Ireland or United Kingdom
land of standing UK United Kingdom
owner Mr. B. Campbell & Mr B Thexton

Soury Gold Cup Winner, The Betfred Supporting The RGT Sprint
Winner, Joe Hardy Memorial Gold Cup Winner, 2008 GBGB
Sprinter of the Year

Boherbradda Kay
IE-JUL-00-BK

Jolly Nikita
IE-JUL-98-F

Hondo Black *
US/IE-JUL-99-BK

EJ's Douglas
US-DEC-90-BD

Hondo Sassafrass
US-DEC-91-BK

Hey Vern
US-FEB-88-RBD

Abigail Girl
US-MAR-85-RF

RB's Ruler
US-NOV-82-BK

Hondo Tormentor
US-DEC-84-BK

Dempsey Duke
IE-FEB-89-BK

Perfect Rhythm
IE-JAN-90-WBD

Cry Dalcash
IE-OCT-91-BD

Princess Nikita
IE-JUL-90-BD

Understood

Dixie's Pixie

Mc's Dutchmaster

Aggie Russo

Unruly

Lacretia

Barry Lyndon

Hondo Checkers

Shanagarry Duke

Willowbrook Peg

Daleys Gold

Rythm N Reason

Greenpark Fox

Dalcash Deal

Manorville Sand

Minnies Nikita

Downing
Economy
Wild Breeze
Miss Westy
Sandy Printer
Mullied
John Denton
Fee Back
Kelly Jones
Skillful
Pacified
Mink Jones
Downing
Blue Betsy
Rooster Cogburn
Hondo Nutmeg

Its Ballyhenry
Glenduff Castle
Echo Spark
Dame Noodle
Linda's Champion
Ballinderry Moth
Knockrour Slave
A Fright
Citizen Supreme
Stern Satoo
Daleys Gold
Sand Melody
Sand Man *
Westmead City
Curryhills Fox
Minnies Selina

Greyhound ID Range

Start 52058

Finish 52058

Update

Race Date Criteria

Process if Last Race Date Occurred Within 6000 Days

Exit

Dog Name: = Boherbradda Mac - 52058

Web Date of Birth: =

Db Date of Birth: = Jun 2005

Records Added = 0

Total Records Added =

Figure 2.3. An example of phenotypic data and pedigree information displayed on the Greyhound-Data (2014) website for an individual Greyhound.

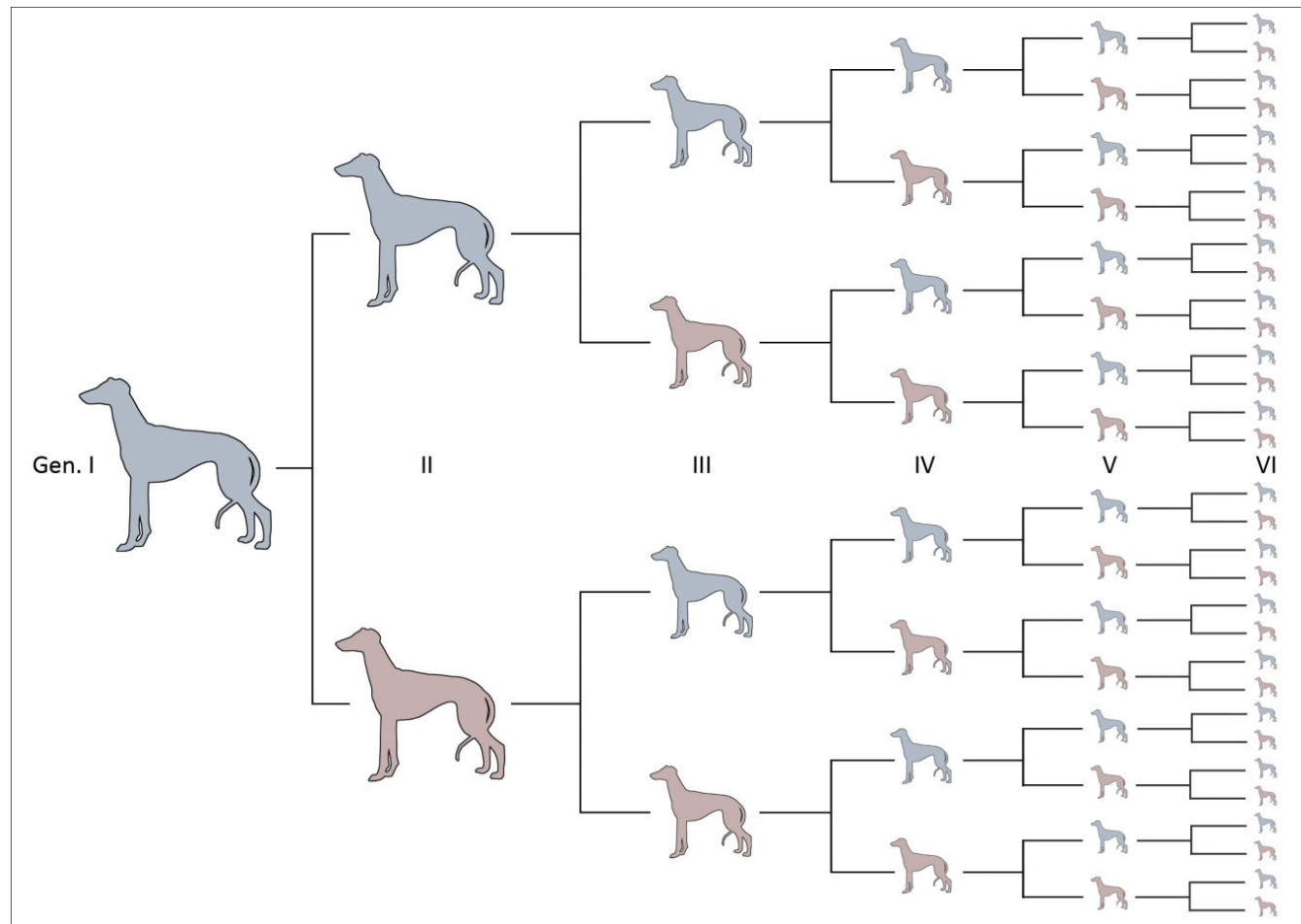


Figure 2.4. Structure of a canine pedigree over six generations (Gen. I to Gen. VI).
Males (sires) = blue; females (dams) = pink. Drawing of Greyhound adapted from www.edwardsart.net, 2014.

The raw phenotypic, pedigree and race performance data were collated into the Microsoft Access database using five relational tables.

2.1.1.1. *Dog Data Table*

The phenotypic 'Dog Data' table consisted of: *GreyhoundID*, a unique identification number assigned to each dog (1 - 73,346); *Name*, the official racing name of the Greyhound; *Sex*, the sex of the dog (M = male, F = female); *SexCode*, integer relating to sex of the dog (1 = male, 2 = female); *DOB*, the Greyhound's date of birth; *Colour*, coat colour of the dog (data was extracted for completeness but was not used in the analysis); *GHDataID*, a unique identification number for each dog, allocated by Greyhound-Data (-1,699,691 to 1,958,364); *Duplicate*, a duplicate field indicating where the software had recorded an individual dog as two or more different dogs. This may occur due to a slight difference in the spelling of a dog's name between race results, or a change in the Greyhound's racing name during its career, although the unique *GHDataID* identifies it as the same dog. A total of 153 duplicate records were identified and flagged by manually changing the *GHDataID* to a negative value, for example *GHDataID* 123456 becomes -123456. In addition, the Dog Data table contained the variable *Link*, a hyperlink to the dog's pedigree chart on the Greyhound-Data website to enable checking of data. An example of information contained in the Dog Data table is displayed in Figure 2.5.

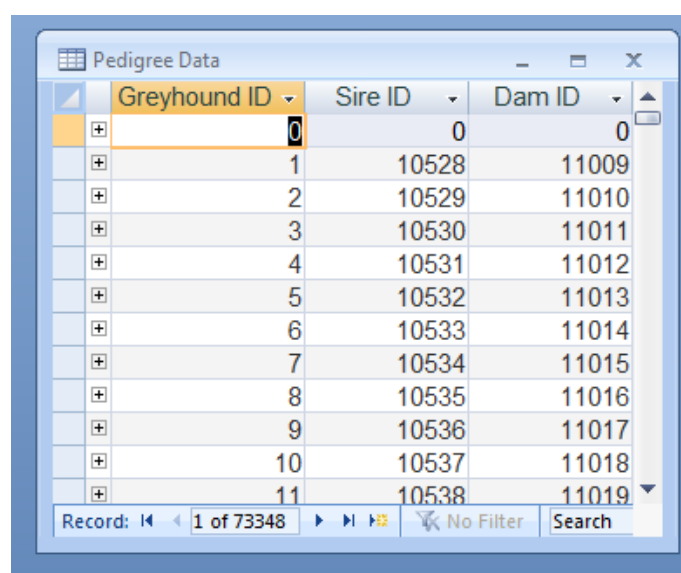
	Greyhound ID	Name	Sex	Sex Code	DOB	Colour	GH Data ID	Duplicate	Link
	0	Unknown					-1		
	1	A Bit Dizzee	F	2	May-2008	Black	1643182		1643182
	2	A Good Excuse	F	2	May-2006	Brindle	1413642		1413642
	3	A Ken Tiger	M	1	Mar-2008	Brindle	1645515		1645515
	4	A Rare Delight	F	2	Sep-2007	Black	1643200		1643200
	5	A Right Druid	M	1	Sep-2006	White & Black	1469066		1469066
	6	A Right One	M	1	Jan-2006	White & Blue	1393677		1393677
	7	Aar Ferrari	M	1	Jan-2007	Black	1539903		1539903
	8	Aaron Trask	M	1	Oct-2007	Fawn	1605647		1605647
	9	Abbey Duchess	F	2	Apr-2006	Black & White	1413019		1413019

Record: 1 of 73346 No Filter Search

Figure 2.5. An example of information displayed in the Dog Data table of the Microsoft Access Database.

2.1.1.2. Pedigree Data Table

The table 'Pedigree Data' consisted of: *GreyhoundID*, as previously defined; *SireID*, which is the *GreyhoundID* of the individual's sire (1 - 73,346); and *DamID*, which is the *GreyhoundID* of the individual's dam (1 - 73,346). An example of information contained in the Pedigree Data table is displayed in Figure 2.6.



The screenshot shows a Microsoft Access window titled 'Pedigree Data'. It displays a table with three columns: 'Greyhound ID', 'Sire ID', and 'Dam ID'. The first row is highlighted in orange and contains the values 0, 0, and 0. Subsequent rows contain numerical values for each column. The bottom of the window shows a status bar indicating 'Record: 1 of 73348' and a search bar.

Greyhound ID	Sire ID	Dam ID
0	0	0
1	10528	11009
2	10529	11010
3	10530	11011
4	10531	11012
5	10532	11013
6	10533	11014
7	10534	11015
8	10535	11016
9	10536	11017
10	10537	11018
11	10538	11019

Figure 2.6. An example of information displayed in the Pedigree Data table of the Microsoft Access Database.

2.1.1.3. Performance Data Table

The table 'Performance Data' consisted of: *ID*, a unique identification number for the table's primary key (1 - 1,882,658); *RaceID*, the unique race identification number allocated by Greyhound-Data (-3,327,215 to 3,331,319); *DogName*, the official racing name of the Greyhound; *GHDataID*, as previously defined;

RacingAge, the age of the dog in months at the time of the race (see Chapter 2.1.3.1); *Position*, the race finish position (1-6); *Rank*, the adjusted race finish position (1-6) according to number of dogs in the race (see Chapter 2.1.3.5); *RootRank*, the square root of *Rank*; *STime*, the Greyhound's split time, which is the time taken in seconds for the Greyhound to reach the finish line for the first time during the race; *Time*, the Greyhound's race finish time in seconds; *Dist*, the approximate finish position (in lengths) relative to the winner; *Speed*, the average speed (m/s) of the dog during the race (see Chapter 2.1.3.4); *TrapNo*, starting trap number (0 - 6); *Posts*, the position (rank) of the dog at each corner of the track during the race (1 - 6); *SP*, racing odds for the dog at the start of the race; and *Comments*, written running remarks regarding the progress and performance of the dog during the race, recorded by officials at the race track. Frequently used Greyhound running remarks and their definitions are displayed in Table 2.1.

Running Remarks	Definition
Ald	Always led
Bmp	Bumped
Brk	Break
Brtdwn	Brought down
Ck	Checked
Chl	Challenged
Clr	Clear
CmAg	Came again
Crmp	Cramped
Crd	Crowded
DNF	Did not finish
EP	Early pace
EvCh	Every chance
FinWll	Finished well
GngWll	Going well
HldOn	Held on
jp	Jumped
KO	Knocked over
Lm	Lame
Lse	Loose
Mid	Middle
msd	Missed
NrLn	Near line
Outp	Outpaced
QAw	Quick away
Rls	Rails
RnIn	Run in
RnOn	Ran on
RnUp	Runner up
SAw	Slow away
SH	Short head
SnLd	Soon led
Stt	Start
Styd	Stayed
Str	Strong(ly)
Swvd	Swerved
th'out	Throughout
Tbl	Trouble
W	Wide

Table 2.1. Commonly used Greyhound running remarks recorded by officials at the race track regarding the progress and performance of the dog during the race, along with their definitions (Greyhound Racing Association Limited, 2014).

For example, written running remarks of 'Bmp1Crd4,Mid' indicate that the Greyhound bumped into another dog at the first bend, experienced crowding with other dogs at the fourth bend, and that it ran in the middle of the track during the race. Running remarks of 'RlsMid,EP,Ld1' indicate that that Greyhound ran between the rails and the middle of the track, started the race well in terms of speed, and was the leading dog at the first bend.

Data for *STime*, *Dist*, *Posts*, *SP* and *Comments* were extracted for completeness but were not used in the analysis. An example of information contained in the Performance Data table is displayed in Figure 2.7.

Performance Data															
ID	RaceID	DogName	GH Data ID	RacingAge	Position	Rank	RootRank	STime	Time	Dist	Speed	TrapNo	Posts	SP	Comments
1	2368369	Not a Trace	1220615	39	1	1	1.00	5.10	29.55 0		16.07	6 1111	9/2		EarlyPace, Led1
2	2368369	Mams Cash	1207644	41	2	2	1.41	5.15	29.75 2		15.97	5 2222	6/4F		Bumped1& 1/4, RanOn
3	2368369	Cutie Pie	1229454	40	3	3	1.73	5.22	29.91 2		15.88	4 4333	8/1		Bumped1
4	2368369	Issy Bee	1225867	50	4	4	2.00	5.14	29.95 0		15.86	1 5444	3/1		Middle, Bumped1
5	2368369	Nellies Choice	1427775	18	5	5	2.24	5.15	30.25 3		15.70	3 6655	5/2		Bumped1& 1/4
6	2368369	Notanothersecond	1442610	21	6	6	2.45	5.22	30.45 2		15.60	2 3566	8/1		SAw, Bumped1, Blk 1/4
7	2368370	Westminsterpearl	1220665	39	1	1	1.00	5.05	29.35 0		16.18	2 1121	5/1		QA w, ALed, Bumped3
8	2368370	Ardmayle Machine	1328511	30	2	2	1.41	5.15	29.59 3		16.05	4 4443	7/2		RanOn
9	2368370	Terry My Pal	1362136	30	3	3	1.73	5.17	29.79 2		15.94	1 3312	2/1F		SAw, Chl&Bumped3
10	2368370	Tullymurry Zulu	1335460	30	4	4	2.00	5.10	29.83 0		15.92	5 2234	4/1		EarlyPace, Bumped3
11	2368370	Retribution	1265725	36	5	5	2.24	5.25	29.93 1		15.87	3 5555	10/1		EveryChance
12	2368370	Call Again	1327388	33	6	6	2.45	5.32	30.05 1		15.81	6 6666	4/1		Hit1H
13	2368371	Firm Handed	1243605	36	1	1	1.00	5.08	29.28 0		16.22	6 3321	1/1F		EarlyPace, Led 3/4
14	2368371	Chase the Dream	1205813	42	2	2.25	1.50	5.20	29.80 6		15.94	1 5555	10/1		SlowAway, RanOn
15	2368371	Cunning Darby	1316756	32	2	2.25	1.50	5.02	29.80 0		15.94	4		7/1	Led1H- 3/4
16	2368371	All Clued Up	1357800	28	4	4.75	2.18	5.17	29.94 1		15.87	5 4444	5/1		Bumped 3/4
17	2368371	Hotdog Jack	1401272	27	5	6	2.45	5.09	29.98 0		15.84	2 2233	6/4		EarlyPace, Bumped 3/4
18	2368372	Townhill Blues	1328515	29	1	1	1.00	5.14	29.55 0		16.07	4 4321	9/4F		Bumped1, Led 3/4
19	2368372	Rain Rush	1366052	28	2	2	1.41	5.06	29.71 2		15.99	3 2552	3/1		BmpRnUp&2, RanOn

Record: 1 of 1882658 No Filter Search

Figure 2.7. An example of information displayed in the Performance Data table of the Microsoft Access Database.

2.1.1.4. *Race Data Table*

The table 'Race Data' contained details of each individual race event, consisting of: *ID*, the identification primary key and unique identification number for each race event (1 - 319,219); *RaceID*, as previously defined; *StadiumID*, the unique identification number of the stadium (1 - 77); *RaceDate*, the date of the race; *RaceTime*, the time that the race took place; *HeatNo*, the heat number (order) of the race during the race meeting; *RaceType*, the type of race (flat, hurdles, handicap, trial); *RaceTypeID*, field not used; *Grade*, the classification grade of the race; *GradeID*, field not used; *Dist*, the race distance in metres (220 to 1,080 m); *NoDogs*, the number of dogs taking part in the race (1 - 6); and *Link*, a hyperlink to the race results displayed on Greyhound-Data, to enable checking of data. An example of information contained in the Race Data table is displayed in Figure 2.8.

2.1.1.5. *Stadium Data Table*

The table 'Stadium Data' contained: *ID*, the unique identification number of the stadium (equivalent to the *StadiumID* previously defined); and *Stadium*, the name of the stadium. An example of information contained in the Stadium Data table is displayed in Figure 2.9.

Race Data													
	ID	RaceID	StadiumID	RaceDate	RaceTime	HeatNo	RaceType	RaceTypeIC	Grade	GradeID	Dist	NoDogs	Link
+	1	2368369	33	01/01/2008	19:45	1	flat_race		A8		475	6	2368369
+	2	2368370	33	01/01/2008	19:58	2	hurdle		H1		475	6	2368370
+	3	2368371	33	01/01/2008	20:11	3	hurdle		H1		475	5	2368371
+	4	2368372	33	01/01/2008	20:24	4	flat_race		A7		475	6	2368372
+	5	2368374	33	01/01/2008	20:49	6	flat_race		S4		640	6	2368374
+	6	2368375	33	01/01/2008	21:03	7	flat_race		A6		475	6	2368375
+	7	2368376	33	01/01/2008	21:16	8	flat_race		A5		475	6	2368376
+	8	2368377	33	01/01/2008	21:29	9	flat_race		S1		640	6	2368377
+	9	2368378	33	01/01/2008	21:42	10	flat_race		A4		475	6	2368378
+	10	2368379	33	01/01/2008	21:55	11	flat_race		A2		475	6	2368379
+	11	2368380	33	01/01/2008	22:07	12	flat_race		A1		475	6	2368380
+	12	2368381	33	01/01/2008	22:20	13	flat_race		S3		640	6	2368381
+	13	2368801	1	02/01/2008	18:49	2	flat_race		A6		470	6	2368801
+	14	2368803	1	02/01/2008	19:22	4	flat_race		A3		470	6	2368803
+	15	2368804	1	02/01/2008	19:37	5	flat_race		A2		470	6	2368804
+	16	2368805	1	02/01/2008	19:53	6	flat_race		S3		590	6	2368805
+	17	2368806	1	02/01/2008	20:09	7	flat_race		A7		470	6	2368806

Record: 1 of 319219 No Filter Search

Figure 2.8. An example of information displayed in the Race Data table of the Microsoft Access Database.

ID	Stadium
1	Bellevue
2	Brough Park
3	Coventry
4	Crayford
5	Hall Green
6	Harlow
7	Henlow
8	Hove & Brighton
9	Kinsley
10	Mildenhall
11	Monmore
12	Nottingham
13	Oxford
14	Pelaw Grange
15	Perry Barr
16	Peterborough
17	Poole
18	Romford
19	Shawfield
20	Sheffield

Figure 2.9. An example of information displayed in the Stadium Data table of the Microsoft Access Database.

2.1.1.6. Relationships Between the Five Tables

The five relational tables of the database are illustrated in Figure 2.10. The phenotypic data table and pedigree data table were linked through the common *DogID* assigned to each Greyhound. The performance data table linked to records contained in the race data table through the *RaceID* assigned to each race event. Records contained in the performance data table were linked to the phenotypic data table through the *GHDataID* assigned to each dog. The race data table and stadium data table were linked through the common *StadiumID* assigned to each race track.

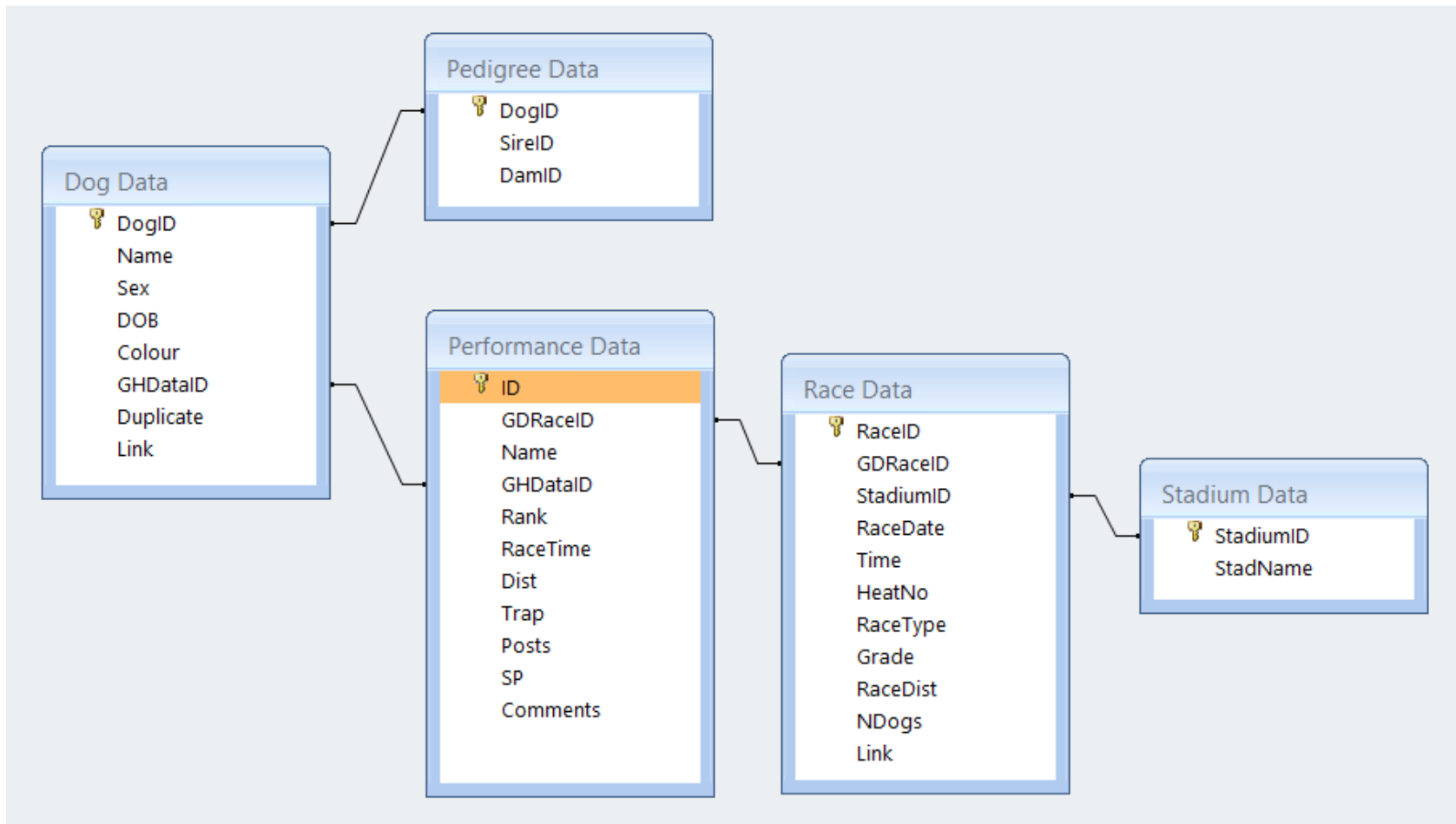


Figure 2.10. Illustration of the five relational tables, Dog Data, Pedigree Data, Performance Data, Race Data and Stadium Data, in the Microsoft Access database. Variables are each defined in Chapters 2.1.1.1 to 2.1.1.5.

2.1.2. Data Cleaning and Quality Control

2.1.2.1. *Data Checking*

The phenotypic, performance and pedigree raw data were cross-checked and cleaned for quality control purposes prior to analysis. With the exception of *Comments*, all non-numerical data were converted to numeric values by recoding, in order to facilitate analysis. Any missing pedigree or phenotypic data were assigned a value of zero, and missing performance data were assigned a value of -99.

In order to check the accuracy of data obtained using the custom software, the data was cross-checked with a smaller quantity of manually collected data from the Greyhound-Data (2014) website. A total of 507,164 race performance records (27% of software-collected data set) for 10,839 individual Greyhounds (21% of software-collected data set) were collected manually by the author from Greyhound-Data (2014), for races taking place during the five-year period from 1st January 2008 to 31st December 2012. The performance data was collated into Microsoft Excel (Microsoft Corporation, 2007b) spreadsheets along with phenotypic and pedigree information for each dog, as previously defined in Chapters 2.1.1.1 to 2.1.1.6. A comparison of the software-collected data and manually-collected data identified no discrepancies.

Additionally, a proportion of the data obtained from Greyhound-Data (2014) was cross-checked with data published by the GBGB (2014) on their website. One race

was selected on an ad hoc basis for each month during the five-year study period, providing a total of 60 races and 360 Greyhounds for data checking. For each of these races and each individual dog, the race event data, performance records, phenotypic and pedigree data were compared by manually cross-checking data published by Greyhound-Data (2014) and the GBGB (2014). No discrepancies were identified between the two sources of data.

Several utility Visual Basic for Applications programs were written in Microsoft Access (G. J. Dockerty, Structural Response Limited, Manchester, 2012) to facilitate data checking and maintenance of the database tables. Modules 'Check_Dog_Table' (Appendix 2a) and 'Check_Pedigree_Table' (Appendix 2b) contained subroutines which checked the Performance Data table against the Phenotypic Data table, and the Phenotypic Data table against the Pedigree Data table, respectively, to determine whether corresponding *GreyhoundID* numbers were present. Module 'Check_Performance_ID' (Appendix 2c) checked the Performance Data table against the Race Data table, to ensure they both contained corresponding values for *RaceID*. Any discrepancies were identified and subsequently investigated and corrected. In the event that an inconsistency in the data could not be corrected or verified, the data was given a negative value and excluded from further analysis.

A 'Check_Pedigree_Data' query (Appendix 3) was performed in Microsoft Access, to cross-check records in the Pedigree Data table and Phenotypic Data table and determine whether any errors in dates of birth were present. It was important for

later analysis stages that a Sire or Dam in the pedigree file was not listed as having a birth year after that of its offspring. Running the 'Check_Pedigree_Data' query identified errors in recorded birth year for several Greyhounds in the data set. Subsequent investigation of individual dog information on Greyhound-Data enabled these errors to be corrected. A small number of Sires and Dams with an unknown date of birth were assigned a random historical birth year to ensure that all offspring receive a birth year after their parents, as the exact parental year of birth is not required for the genetic analysis. Running the query for a second time found no further errors in the pedigree data.

2.1.2.2. Greyhound Stadium (StadiumID)

The majority of Greyhound performance raw data consisted of race results for the 26 GBGB-licensed race tracks in operation in the UK during the period of data collection. The data set, however, also contained performance data for an additional 51 Independent (non-GBGB) British tracks and international Greyhound stadiums. British racing Greyhounds commonly start their racing career in Ireland before transferring to the UK. Consequently, British Greyhounds frequently possess a number of historical race performance results for races that took place in Ireland, or another country of origin. Rules of racing and the format of racing varies between different countries and regulatory bodies. Furthermore, there is no central regulatory body for the Independent sector of the British Greyhound racing industry. Consequently, there is variation in racing rules and regulation between Independent tracks in the UK. Data from Independent British tracks and

international Greyhound stadiums were therefore excluded from further analysis, leaving only performance data from the 26 GBGB-licensed British race tracks in operation during the period of data collection (2008 to 2012).

2.1.2.3. Number of Dogs in the Race (Ndogs)

The majority of Greyhound races on GBGB tracks consist of five or six dogs. All races with records of less than four competing Greyhounds were excluded from the data set.

2.1.2.4. Type of Race (RaceType)

To ensure only flat races starting from a level-break ('scratch races') were included in the analysis, all trials, races over hurdles and handicap races were excluded from the data set.

2.1.2.5. Race Finish Position (Rank)

In the UK, the rank position is a score from one to six based on the order of finishing the race, with the winning Greyhound receiving a rank of one (first). Race performance results with a recorded rank position greater than six were therefore excluded from the data set.

2.1.3. Additional Calculations Performed on the Phenotypic and Performance Raw Data

2.1.3.1. Greyhound Racing Age (*RacingAge*)

Additional calculations were performed on the raw data to determine *RacingAge*, the age of the dog in months at the time of the race. For every Greyhound and each of its race performance results, the variable *RacingAge* was calculated as follows:

RacingAge (in months) =

(month of race - month of birth) + [(year of race - year of birth) x 12]

2.1.3.2. Month-Year-Stadium Combination (*MYStad*)

Due to the large data set and computational limitations of the software used in the present study, it was not possible to examine the effect of local environmental conditions on Greyhound race performance in terms of variation between individual race events, *RaceID* (1 - 319,219), or combinations of stadium and race date, *StadDate* (47,450 levels), as both these variables exceeded the maximum number of levels permitted in the model. In order to investigate the influence of season, climatic conditions and stadium on Greyhound racing performance, a combination of race month, race year and stadium, *MYStad*, was therefore produced for each race performance result (n = 1,711,489) in the data set. The following formula was used to calculate the variable *MYStad*:

$$MYStad = [(racemo \times 100 + raceyr) \times 100] + StadiumID$$

Where *racemo*, the month of the race event (1 - 12); *raceyr*, the year of the race event (2008 - 2012); and *StadiumID*, the numerical identification code of the stadium of the race (1 - 26). This resulted in the creation of 1,509 unique *MYStad* values in total.

Limitations of using the *MYStad* variable in further analysis of the data are that it does not take into consideration the variations in environmental conditions between different race days in a particular month, or between race events at a particular stadium on a specific date.

2.1.3.3. *Type of Race (RaceType)*

The race distances and number of bends encountered during the race varied considerably both within and between tracks. Performance data were therefore grouped into four different Race Types: *RaceType 1*, consisted of two-bend 'sprint' races over distances between 220 m and 305 m; *RaceType 2*, consisted of four-bend 'middle-distance' races over distances between 375 m and 515 m; *RaceType 3*, consisted of six-bend 'stayer' races between 540 m and 700 m; and Race Type four consisted of 'marathon' races over 700 m.

2.1.3.4. Average Speed (*Speed*)

Additional calculations were performed on the raw data to determine the average speed (m/s) of the dog during the race. For every Greyhound and each of its race performance results, the variable *Speed* (m/s) was calculated as follows:

$$\text{Speed (m/s)} = \frac{\text{Race distance (m)}}{\text{Race finish time (s)}}$$

Greyhounds are capable of racing at speeds up to 18 m/s (Dobson *et al.*, 1988), but they are able to maintain this speed for only the first 250 m of a race. Average speeds considerably greater than this are therefore likely to reflect an error in the recorded race time. Extremely slow racing speeds were occasionally observed, where the Greyhound is likely to have sustained an injury or collided with another dog during the race. For races over the most common racing distance of 480 m ($n = 481,882$), the mean, maximum and minimum speeds in the raw data set were 16.1 m/s, 17.5 m/s and 11.6 m/s, respectively. Performance results over all race distances ($n = 1,882,658$) that contained average speeds of less than 10 m/s or greater than 20 m/s ($n = 170,517$) were therefore excluded from the data set.

2.1.3.5. Adjusted Rank Position (*AdjRank*)

The Greyhound rank position was adjusted in order to correct the results for races with different numbers of dogs, as described by Taubert *et al.* (2007). The adjusted rank position, *AdjRank*, was calculated using the following formula:

$$5 \left(\frac{R-1}{N-1} \right) + 1$$

(Taubert *et al.*, 2007)

Where R is the rank position of the Greyhound and N is the total number of dogs taking part in the race. This formula corrects the rank positions for races with less than six dogs taking part, therefore enabling comparisons between races with different numbers of participants. For example, a Greyhound finishing third in a race with a total number of five dogs taking part would be assigned an adjusted rank position of 3.5, and the Greyhound finishing last (fifth) would receive an adjusted rank position of six.

2.1.4. The Final Data Set

Following comprehensive data cleaning, cross-checking and quality control, the final data set (Appendix 1) contained a total of 1,711,489 individual race performance records for 50,452 individual Greyhounds, with a total of 73,344 Greyhounds in the Pedigree Data table. Overall, 22,892 Greyhounds in the pedigree file did not have any performance records associated with them. These dogs did not take part in races within the study period of 1st January 2008 to 31st December 2012, but were relatives (within a six-generation pedigree) of the 50,452 Greyhounds with performance records.

2.1.5. Creation of the Pedigree File for Genetic Analysis

The pedigree file for analysis was created using the following space-delimited four-column format, with one row for each individual Greyhound:

NumIdInd *NumIdSire* *NumIdDam* *BirthYrInd*

Where *NumIdInd*, *NumIdSire* and *NumIdDam* are the unique numerical identification codes assigned for the individual Greyhound (1 - 73,344), its Sire (1 - 73,344) and its Dam (1 - 73,344) respectively, and *BirthYrInd* is the birth year of the individual Greyhound (1953 to 2011). Each Sire and Dam also appeared with their own row, as did the maternal and paternal grandparents, great-grandparents, great-great-grandparents and great-great-great-grandparents. Unknown parents were assigned a numerical identification code of zero (0) (Gilmour *et al.*, 2009). For example, the first 11 rows of the Greyhound pedigree file are displayed below:

```
0 0 0 1953
1 10528 11009 2008
2 10529 11010 2006
3 10530 11011 2008
4 10531 11012 2007
5 10532 11013 2006
6 10533 11014 2006
7 10534 11015 2007
8 10535 11016 2007
9 10536 11017 2006
10 10537 11018 2006
```

2.1.6. General Analysis and Statistical Methods

General statistical analysis, and analysis to determine the environmental effects and covariates to consider in the final genetic analyses of Greyhound racing performance, were performed using the software SAS, version 9.3 (SAS Institute Inc., 2002-2010). The variance components and breeding values were estimated using the freely available software package DMU, version 6, release 5.2 (Madsen and Jensen, 2013). The pedigree file and Greyhound phenotypic and performance data file were exported as two separate text files for input to SAS and DMU. Graphical representations of the data were performed using the software packages SAS, version 9.3 (SAS Institute Inc., 2002-2010) and Microsoft Office Excel (Microsoft Corporation, 2007b).

2.2. Genome-Wide Association Study (GWAS) of Stress Fracture Injuries in the Racing Greyhound

2.2.1. Case and Control Selection

The inclusion criteria for case (affected) samples were: Greyhounds that had taken part in a total of 50 races or less and had sustained a stress fracture of the tarsal, metacarpal (MC) or metatarsal (MT) bones of the distal limb during racing, trialling or schooling at the stadium, as diagnosed by the Greyhound track veterinary surgeon in attendance. Case samples were collected from Greyhounds that regularly raced at one GBGB-licensed Greyhound stadium in the UK, and had either been euthanased at the stadium due to serious injury, or had previously sustained a stress fracture and were returned to racing or retired.

The inclusion criteria for control (unaffected) samples were: Greyhounds that had taken part in over 70 races in total, were healthy and had never sustained a fracture of any type, as reported by the owner or trainer of the dog and confirmed by the Greyhound track veterinary surgeon. Control Greyhounds were recruited from the same GBGB-licensed Greyhound stadium as case Greyhounds. All control Greyhounds included in the study were examined by a veterinary surgeon (the author) immediately prior to sample collection, and confirmed as being in good health.

Previous analysis of the British GBGB-licensed Greyhound population over a five year period from 2008 to 2012 ($n = 50,452$) found that 76% ($n = 38,344$) of

Greyhounds had taken part in a total of 50 races or less, and only 12.3% (n = 6,206) of Greyhounds had taken part in more than 70 races (Figure 3.1). Consequently, a maximum of 50 races was selected for case samples in order to include only those Greyhounds that sustained a stress fracture during the earlier stages of their racing career, which is suggestive of a strong genetic component. Seventy races was selected as a minimum value for control samples in order to include only those Greyhounds with a relatively long racing career and no history of bone fractures, therefore implying the dogs had good bone strength and potentially a lower genetic risk of stress fracture.

2.2.1.1. Sample Collection

Within a multicellular organism, each different cell type contains identical DNA (Alberts *et al.*, 2002). Animal DNA samples can be obtained from a wide range of biological materials, including tissue, blood, saliva, hair and semen. Several types of biological samples were collected from case and control Greyhounds in the present study, in order to obtain samples of their DNA.

All samples were collected with the full informed consent of the Greyhound owner, or of the Greyhound trainer responsible for the care of the dog and acting on behalf of the owner. Each Greyhound owner or trainer was provided with an information sheet (Appendix 4 and Appendix 5) detailing the study, and completed a written consent form (Appendix 6 and Appendix 7) giving permission for sample collection, storage and use in scientific research. All tissue samples were collected

from Greyhounds that had sustained an injury and were euthanased at the stadium by the attending track veterinary surgeon for reasons unrelated to the present study. The research project received ethical approval from the University of Liverpool Veterinary Research Ethics Committee.

2.2.1.2. Muscle Tissue Sample Collection

Muscle tissue samples were collected from 51 case (affected) Greyhound cadavers (33 males and 18 females) that had sustained a stress fracture of the tarsus. Time restrictions prevented immediate post-mortem collection of Greyhound saliva, blood or muscle tissue samples by the track veterinary surgeon. The Greyhound cadaver was frozen at -20 degrees Celsius (°C) immediately following euthanasia, therefore enabling collection of tissue samples at a later date.

The Greyhound cadaver was collected from the stadium and transported to the School of Veterinary Science at the University of Liverpool. Prior to sample collection, the cadaver was thawed in a cold store for five to seven days. Phenotypic data consisting of the Greyhound's racing name, ear tattoo, sex, weight and colour were recorded, and the tarsal fracture was confirmed by palpation and/or dissection of the affected limb. Samples of biceps femoris muscle tissue were collected from the dog and immediately stored at -80°C until required for further processing.

Of the total 51 muscle tissue samples, 45 were collected as part of a previous study (Hercock, 2010) and six were obtained by the author.

2.2.1.3. *Skin Tissue Sample Collection*

The track veterinary surgeon was provided with skin biopsy kits for the collection of Greyhound skin tissue samples. This was to test an alternative method of tissue sample collection that could be performed quickly and easily by the track veterinary surgeon during the immediate post-mortem period. In total, skin tissue samples were collected from two case Greyhound cadavers (one male and one female) that had sustained a stress fracture of the tarsus. Immediately following euthanasia, a suitable skin biopsy site was selected and the overlying hair trimmed using scissors. The biopsy site was not surgically prepared or sterilised. A full-thickness skin biopsy sample was collected by the track veterinary surgeon using a sterile disposable five millimetre (mm) biopsy punch, and placed into a two millilitre (ml) screw-cap tube containing 500 microlitres (μ l) DNAgard Tissue solution (Biomatrica, USA), ensuring that the tissue sample was fully submerged. The procedure was repeated, so that two skin biopsy samples were collected from each dog. The 2 ml screw cap tubes were then wrapped in foil to protect the samples from light, and stored upright at room temperature until required for further processing. Phenotypic data consisting of the Greyhound's racing name, ear tattoo, sex and details of the injury were recorded. The date of birth, pedigree and historical race performance data for each Greyhound were obtained from the online resource Greyhound-Data (2014).

2.2.1.4. *Saliva Sample Collection*

Blood sampling is considered an invasive procedure, and a home office licence is required for collection of blood samples from live dogs for research purposes in the UK. Saliva collection, however, is considered a non-invasive procedure and does not require a licence.

Saliva samples were collected from 38 case Greyhounds (21 males and 17 females) that had previously sustained a stress fracture of the tarsus (30 dogs), metacarpus (two dogs) or metatarsus (six dogs), and were returned to racing or retired. In addition, saliva samples were collected from 146 healthy control Greyhounds (80 males and 66 females). Due to GBGB regulations restricting the sampling of Greyhounds while at the stadium for racing or trialling, all saliva samples were collected from Greyhounds at their residential kennels on non-race days. A number of Greyhounds were sampled from each of the 15 racing kennels associated with the GBGB-licensed Greyhound stadium.

Saliva samples were collected using Oragene®-Animal OA-400 saliva collection kits, (Oragene®, DNA Genotek Inc., Ontario, Canada), which enable quick, easy, non-invasive collection of high-quality and high-quantity DNA, providing similar DNA yields to bloods samples (Iwasiow *et al.*, 2009). Additionally, DNA samples collected using Oragene®-Animal saliva kits remain stable at room temperature for up to six months, enabling ease of sample collection, transport and storage. The

Oragene®-Animal kit consisted of two sponges and a collection tube containing the Oragene®-Animal buffer solution.

All saliva samples were collected by the author to ensure consistency of the sampling procedure. To minimise contamination or dilution of the sample, the Greyhound was not permitted to eat for 30 minutes or drink for 10 minutes prior to sample collection. Saliva samples were collected according to the manufacturer's instructions. Briefly, the two Oragene®-Animal sponges were held together and placed into the Greyhound's mouth at the right or left cheek pouch, and saliva was collected for 30 s by moving the sponges and absorbing the saliva where it naturally pools. This procedure was repeated on the other side of the Greyhound's mouth for a further 30 s. The two sponges were then removed from the dog's mouth, ensuring that the teeth and lips were not scraped during removal, and placed into the collection tube. The capped tube was then shaken vigorously for 10 s, labelled, and stored upright at room temperature until required for further processing. Nitrile gloves were worn throughout the sample collection process.

A questionnaire (Appendix 8) was used to obtain phenotypic data from the Greyhound owner or trainer. This data consisted of the Greyhound's racing name, ear tattoo, sex, place of birth, weight, trainer, medical and injury history. The date of birth, pedigree and historical race performance data for each Greyhound were obtained from the online resource Greyhound-Data (2014).

2.2.2. Sample Processing

All DNA extraction procedures were performed by the author at the Centre for Integrated Genomic Medical Research (CIGMR) at the University of Manchester (Manchester, UK) and the Institute of Integrative Biology at the University of Liverpool (Liverpool, UK).

2.2.2.1. *DNA Extraction from Muscle Tissue Samples*

DNA was extracted from muscle tissue samples using Qiagen DNeasy Blood and Tissue Mini Kits (Qiagen UK Limited, Crawley, West Sussex, UK) according to the manufacturer's instructions. Briefly, a small section of the frozen muscle tissue sample was thawed to room temperature. A cube of tissue measuring four mm by four mm was cut using a scalpel, and then further cut into small pieces to enable efficient lysis. The sample was placed into a labelled 1.5 ml microcentrifuge tube. 180 µl Buffer ATL (Qiagen, UK) and 20 µl proteinase K (Qiagen, UK) were added to the tube and mixed by vortexing. The sample was incubated in a water bath at 56 °C overnight until the tissue was completely lysed.

At this stage of the protocol, the optional step of adding 4 µl RNase A (100 mg/ml) was tested in a small number of samples (n = 8) using Riboshredder™ RNase Blend (Epicentre, Madison, Wisconsin), in order to obtain RNA-free genomic DNA. The addition of Riboshredder™ RNase Blend was found to make no difference to the final DNA concentration or quality of the sample, as measured using a Nanodrop® spectrophotometer (Thermo Scientific, Delaware, USA). The average DNA yields

were 42.8 ng/μl and 43.1 ng/μl for DNA extractions performed with and without the addition of Riboshredder™ RNase Blend, respectively. Consequently, the optional step of addition of RNase A was excluded from the protocol.

Following tissue lysis, the sample was vortexed for 15 s. 200 μl Buffer AL (Qiagen, UK) was added to the sample and mixed thoroughly by vortexing. 200 μl 100% ethanol was added to the sample, and mixed immediately by vortexing. Using a sterile 3 ml pastette, the resulting mixture was transferred to a labelled DNeasy Mini spin column (Qiagen, UK) placed in a 2 ml collection tube, and centrifuged at >6,000 *g*-force (*g*), or 8,000 rotations per minute (rpm), for one minute in a microcentrifuge. The flow-through and collection tube were discarded. The DNeasy Mini spin column (Qiagen, UK) was placed in a new 2 ml collection tube. 500 μl Buffer AW1 (Qiagen, UK) was added, and the sample centrifuged at >6,000 *g* (8,000 rpm) for one minute in a microcentrifuge. The flow-through and collection tube were discarded. The DNeasy Mini spin column (Qiagen, UK) was placed in a new 2 ml collection tube. 500 μl Buffer AW2 (Qiagen, UK) was added, and the sample centrifuged at 20,000 *g* (14,000 rpm) for three minutes in a microcentrifuge, to dry the DNeasy membrane. The flow-through and collection tube were discarded. The DNeasy Mini spin column (Qiagen, UK) was placed in a new 2 ml collection tube, and 200 μl Buffer AE (Qiagen, UK) was pipetted directly onto the DNeasy membrane. The sample was incubated at room temperature for one minute, and then centrifuged at >6,000 *g* (8,000 rpm) for one minute in a microcentrifuge to elute. This elution step was repeated once to enable maximum DNA yield. The 2 ml collection tube containing the first eluate was reused for the

second elution step, and 200 µl Buffer AE (Qiagen, UK) was pipetted directly onto the DNeasy membrane. The sample was incubated at room temperature for one minute, and then centrifuged at >6,000 *g* (8,000 rpm) for one minute in a microcentrifuge. The total eluate was transferred to a labelled Cryo.s™ 2 ml cryogenic storage vial (Greiner Bio-One Limited, Stonehouse, UK), and stored at -20 °C until required for further processing.

2.2.2.2. *DNA Extraction from Skin Tissue Samples*

DNA was extracted from skin samples using a modified protocol for DNA recovery from a tissue sample stored in 500 µl DNAgard Tissue solution (Biomatrica, USA). Briefly, the skin tissue sample was removed from the storage tube containing DNAgard Tissue solution. The skin tissue sample was cut into small pieces to enable efficient lysis, then placed into a labelled 2 ml microcentrifuge tube. 400 µl Buffer ATL (Qiagen, UK) and 40 µl proteinase K (Qiagen, UK) was added to the tube and mixed by vortexing. The sample was incubated in a water bath at 56 °C overnight until the tissue was completely lysed.

The sample was then vortexed for 15 s. 400 µl Buffer AL (Qiagen, UK) was added to the sample and mixed thoroughly by vortexing. 400 µl of 100% ethanol was added to the sample, and mixed immediately by vortexing. Using a sterile 3 ml pastette, the resulting mixture was transferred to a labelled DNeasy Mini spin column (Qiagen, UK) placed in a 2 ml collection tube. The remaining steps in the protocol

are the same as those previously described for DNA extraction from muscle tissue samples (Chapter 2.2.2.1).

2.2.2.3. *DNA Extraction from Saliva Samples*

A modification of the manufacturer's protocol (DNA Genotek, Canada) was used to extract DNA from the saliva samples collected using Oragene® Animal kits, to enable processing of the entire sample (approximately 2 ml) rather than a 500 µl aliquot. Briefly, the teal coloured cap of the sample tube was replaced with the supplied purple cap. The samples were mixed in the tube by inverting several times, and then incubated in a water bath at 50 °C for a minimum of one hour. The collection sponges were pressed against the side of the collection tube to release additional liquid contained in the sponge. 1/25th volume (40 µl per 1 ml of sample) of Oragene® DNA Purifier (DNA Genotek, Canada) was added to the tube and vortexed briefly to mix. The samples were incubated on ice for 10 minutes and then centrifuged at room temperature at 4,500 rpm (4,000 g) for 17.5 minutes in a Jouan centrifuge. The clear supernatant was transferred to a new labelled 15 ml tube using a sterile 3 ml pastette, ensuring that the pellet containing impurities remained undisturbed. The pellet was then discarded. Five-molar (M) NaCl (60 µl per 1 ml of sample) was added to the supernatant to ensure efficient recovery of DNA. An equal volume to the sample of room temperature 100% ethanol was added to the tube and mixed by inverting 10 times, then incubated at room temperature for 10 minutes. Samples were centrifuged at room temperature for 17.5 minutes at 4,500 rpm (4,000 g) in a Jouan centrifuge. The supernatant was

removed using a sterile 3 ml pastette and discarded, ensuring that the pellet containing the DNA remained undisturbed. 1 ml 70% ethanol was added to the tube and left at room temperature for one minute. The tube was gently swirled and then the ethanol completely removed using a sterile 3 ml pastette. The DNA pellet was dried at room temperature for 15 to 20 minutes, then re-suspended in 300 µl TE buffer (10mM Tris, 1mM EDTA) and vortexed for 30 s. Samples were incubated in a water bath at 50 °C for one hour with occasional vortexing. The rehydrated DNA was transferred to a labelled Cryo.s™ 2 ml cryogenic storage vial (Greiner Bio-One Limited, Stonehouse, UK), and stored at -40 °C until required for further processing.

2.2.3. Quantification of DNA

DNA quantification was performed using the Quant-iT™ dsDNA Broad-Range Assay Kit (Invitrogen™, Life Technologies, Paisley, UK) and measured using a Qubit® fluorometer (Invitrogen™, Life Technologies, Paisley, UK), according to the manufacturer's instructions.

2.2.4. General Analysis and Statistical Methods

Genotyping data was analysed using the freely available, open-source whole-genome association analysis software PLINK, version 1.07 (Purcell *et al.*, 2007; Purcell, 2009). The position of associated SNPs on the chromosome and potential causative genes were identified from the CanFam2.0 and CanFam3.1 canine genome assembly using the Ensembl online public database (Cunningham *et al.*,

2015; Ensembl, 2015). Graphical representation of the data was performed using the software packages Haploview, version 4.2 (Barrett *et al.*, 2005) and R, version 3.1.1 (The R Foundation for Statistical Computing, 2014).

2.3. Salivary Cortisol and Heart Rate as Measures of Stress in the Racing Greyhound

2.3.1. Greyhound Selection

A total of 59 healthy Greyhounds (32 males and 27 females) racing at an Independent (non-GBGB licensed) British Greyhound stadium were recruited to participate in the study. This was an opportunistic sample that included all Greyhounds whose owner or trainer had consented to take part. All dogs were trained for racing and had raced competitively at a commercial track. Each Greyhound included in the study was examined by a veterinary surgeon (the author) as part of the required pre-race veterinary inspection as specified in The Welfare of Racing Greyhounds Regulations 2010 (England), and confirmed as healthy and fit to race.

Samples and data were collected with the full informed consent of the Greyhound owner, or of the Greyhound trainer responsible for the care of the dog and acting on behalf of the owner. In the Independent sector of the Greyhound industry, the trainer and owner of the Greyhound are frequently the same person. Each Greyhound owner or trainer was provided with an information sheet (Appendix 11) detailing the study, and completed a written consent form (Appendix 6) giving permission for sample collection, storage and use in scientific research. The General Manager of the Independent Greyhound stadium gave their full informed

consent for the stadium to participate in the study, and permitted the collection of samples on the premises both before and after racing.

2.3.2. Sample Collection

The rules and regulations of the GBGB restrict the collection of samples from Greyhounds present at licensed race track premises. Consequently, sample collection took place at an Independent (non-GBGB-licensed) Greyhound stadium during two consecutive race meetings held one week apart, in December 2013.

Independent race tracks are required to provide kennels for a minimum of 20% of the total number of Greyhounds present at the stadium at any one time for trialling or racing (The Welfare of Racing Greyhound Regulations, 2010). There is, however, no requirement for pre-race kennelling of the Greyhound. Consequently, on arrival at the stadium Greyhounds remained either in their owner or trainer's van, the car park, paddock area or stadium kennel, depending on the preference of the owner or trainer. Adhering to the usual race meeting procedures, Greyhound owners and trainers presented their dog for race registration and the required pre-race veterinary examination, approximately 15 to 20 minutes before the start of the Greyhound's race. All Greyhounds participating in the study were used to frequent handling and were familiar with a procedure of pre-race clinical examination by a veterinary surgeon, which included inspection of the mouth and teeth. Dogs were lightly restrained during sample collection by their owner or trainer using a neck

collar and lead only, as they would normally be restrained during the pre-race and post-race periods at the Greyhound stadium to prevent escape.

All samples and data were collected by a veterinary surgeon (the author) and a team of 14 University of Liverpool Veterinary Science students, trained in the sampling procedures by the author.

2.3.2.1. *Saliva Sample Collection*

Canine cortisol levels can be measured in a wide range of biological samples, including saliva (Beerda *et al.*, 1998; Bergeron *et al.*, 2002; Dreschel and Granger, 2009; Hekman *et al.*, 2012; Kobelt *et al.*, 2003; Vincent and Michell, 1992). Saliva collection is considered a non-invasive procedure and does not require a home-office licence. Canine salivary cortisol levels are highly correlated with plasma cortisol (Beerda *et al.*, 1996; Vincent and Michell, 1992), and can therefore be used as a measure of HPA activity.

Saliva samples were collected from all Greyhounds using Salimetrics® Children's Swabs (SCS) (Salimetrics® Europe Limited, Suffolk, UK). The SCS is a highly absorbent, non-toxic, inert polymer sponge measuring 8 mm by 125 mm, and is durable and very resistant to chewing, making it suitable for saliva collection from dogs. The volume of saliva sample recovered from the SCS is approximately 200 µl to 1,000 µl (Salimetrics, 2013), and the swabs are approved for collection of saliva for analysis of cortisol.

Previous studies have used salivary stimulants such as citric acid (Beerda *et al.*, 1998; Bergeron *et al.*, 2002; Kobelt *et al.*, 2003; Pastore *et al.*, 2011) to induce salivation in dogs, in order to increase the volume of saliva collected and ensure an adequate quantity for cortisol measurement. Brorrsson *et al.* (2014) reported that stimulation of salivation using citric acid had no effect on salivary cortisol levels in humans. Other studies, however, reported that this artificially increases cortisol concentrations in human (Schwartz *et al.*, 1998) and canine (Dreschel and Granger, 2009) saliva samples. Additionally, Dreschel and Granger (2009) report that the use of beef-flavoured collection materials results in unpredictable variability in salivary cortisol concentrations in dogs. Therefore, in order to minimise the risk of artificially affecting salivary cortisol levels, and to ensure that the dog was not required as part of the study to ingest a substance pre-race that may potentially affect its racing performance, no salivary stimulants were used in the present study when collecting saliva samples from Greyhounds. The SCS sampling device contained no food-based additives that may have interfered with subsequent cortisol measurement of saliva samples (Dreschel and Granger, 2009).

Koyama *et al.* (2003) found that salivary cortisol secretion in dogs does not follow a circadian rhythm. Other studies, however, report considerable variations in canine blood cortisol concentrations during a 24-hour period (Kolevska *et al.*, 2003). All samples in the present study were collected between the hours of 19:30 to 23:00, reducing the effect of potential temporal variation or circadian deviation on salivary cortisol concentrations.

A saliva sample was collected from each Greyhound both before and after racing. The pre-race saliva sample was collected 15 to 20 minutes before the start of the Greyhound's race. The post-race sample was collected immediately after the Greyhound was walked off the racetrack, within five minutes of the end of the race.

To minimise contamination or dilution of the saliva sample, the Greyhound was not permitted to eat for 30 minutes or drink for 10 minutes prior to sample collection. Saliva samples were collected according to the manufacturer's instructions (Salimetrics® Europe Limited, Suffolk, UK). Holding the SCS securely at one end, the other end of the swab was placed into the dog's mouth at the right or left cheek pouch, and saliva was collected for a minimum of 90 s by moving the swab and absorbing the saliva where it naturally pools in the cheek pouch and under the tongue. Using the same SCS, the procedure was repeated on the other side of the dog's mouth for a further 90 s, or until the lower third of the swab was saturated. For each dog, saliva samples were collected within four minutes to prevent elevation of salivary cortisol concentrations due to handling (Kobelt *et al.*, 2003). The SCS was removed from the dog's mouth, ensuring that the teeth and lips were not scraped during removal, and the saturated end was placed into the upper tube insert of a Salimetrics® Swab Storage Tube (SST) (Salimetrics® Europe Limited, Suffolk, UK). The dry section of the SCS was cut off using scissors and discarded, and the remaining saturated SCS was folded to fit into the upper tube insert of the SST. The tube was capped tightly to prevent evaporation, labelled and refrigerated immediately at 4 °C for the remainder of the race meeting. The

samples were transferred in an ice box to a freezer within three hours, and stored at -20 °C until required for further processing. Nitrile gloves were worn throughout the sample collection process.

2.3.2.2. *Heart Rate Measurement*

The heart rate (HR) of each Greyhound was assessed both before and after racing by cardiac auscultation using a stethoscope, over a period of one minute. The pre-race HR was measured 15 to 20 minutes before the start of the Greyhound's race. The post-race HR was measured immediately after the Greyhound was walked off the race track, within five minutes of the end of the race. The procedure of cardiac auscultation using a stethoscope may in itself cause stress in some dogs and result in an increase in HR. Consequently, the HR was measured with minimal restraint and handling of the Greyhound to minimise this effect.

For each dog, saliva sample collection and heart rate measurement occurred simultaneously both before and after racing. Additionally, saliva samples and heart rate measurements were collected at the same time from each Greyhound taking part in the race, both before and after racing, using a team of 14 University of Liverpool Veterinary Science students.

2.3.2.3. *Phenotypic Data*

Greyhound owners and trainers were asked to provide information about the dog and their journey to the Greyhound stadium that day, including: racing name; ear

tattoo; sex and age of the dog; post code of the location the dog had travelled from (in order to calculate journey distance); and the journey time. For each Greyhound, additional information was collected from the printed Racing Programme, including: race number; time of the race; race distance; starting trap number; and number of dogs in the race (Appendix 10). The Greyhounds included in the present study were non-GBGB licensed dogs, and their pedigree and historical race performance data were unavailable.

2.3.3. Cortisol Enzyme Immunoassay (EIA)

Sample processing and measurement of cortisol concentrations were performed at the Department of Biological Sciences at the University of Chester (Chester, UK) (R. Coleman). Prior to analysis, the storage tubes containing the swab were thawed to room temperature and centrifuged for 15 minutes at 3,000 rpm (1,500 *g*), to obtain the saliva sample. Cortisol concentrations were measured in all saliva samples using an enzyme immunoassay (EIA), modified from a protocol described previously (Smith and French, 1997). Briefly, cortisol antibody [R4866, raised against a steroid-conjugated bovine serum albumin (BSA) in rabbit] was diluted in 0.05M EIA coating buffer (1.59 g Na₂CO₃; 2.93 g NaHCO₃; 1,000 ml dH₂O; pH 9.6) to a dilution of 1:12,000. 50 µl of antibody dilution was added to all wells of the 96-well microtitre plate, except the Non-Specific Binding (NSB) wells. 50 µl coating buffer or dH₂O were added to the NSB wells, to control for non-antibody binding. The edges of the plate were tapped lightly to ensure the bottom of the wells were

evenly coated with antibody. The plates were covered with an adhesive plate sealer and sealed completely, then incubated at 4 °C for 12 to 18 hours.

The plates, samples and buffers were brought to room temperature. The plates were washed four times with wash solution using a Dynatech Ultrawash Plus automatic plate washer (Dynex Technologies Limited, West Sussex, UK), then blotted dry on paper towels. 50 µl of 0.1M EIA phosphate buffered saline (PBS) (5.42 g NaH₂PO₄; 8.66 g Na₂HPO₄ [anhydrous]; 8.7 g NaCl; 1.0 g BSA [Radioimmunoassay (RIA) Grade Bovine Albumin]; 1,000 ml dH₂O; pH 7.0) was immediately added to each well of the plate, and the plates were resealed. Saliva samples were diluted to 1:16 in dH₂O. 50 µl of prepared standards, pool controls, water and samples were added to the appropriate wells. The steroid conjugate, cortisol-horseradish peroxidase (HRP), was diluted in EIA PBS to a dilution of 1:22,000. Immediately after the addition of samples, 50 µl cortisol-HRP conjugate dilution was added to all wells of the plate. The plates were sealed and incubated at room temperature for two to three hours in the dark.

The plates were washed four times with wash solution as previously, then resealed. EIA substrate solution was prepared using 0.05M EIA citrate buffer (9.61 g citric acid [anhydrous]; 1,000 ml dH₂O; pH 4.0); 40mM EIA ABTS [2,2'-Azino-bis(3-ethylbenzthyloline-6-sulfonic acid) Diammonium Salt] (0.329 g ABTS; 15.0 ml dH₂O; pH 6.0); and 0.05M EIA H₂O₂ (500 µl 8M H₂O₂; 7.5 ml dH₂O), as displayed in Table 2.2.

Number of Plates	Volume of Citrate Buffer (ml)	Volume of ABTS (μ l)	Volume of H ₂ O ₂ (μ l)
2	25.0	250.0	80.0
4	50.0	500.0	160.0
6	75.0	750.0	240.0

Table 2.2. Preparation of Enzyme Immunoassay (EIA) substrate solution.

100 μ l EIA substrate solution was added to all wells of the plates. The plates were sealed tightly and then shaken continuously until colour developed to an optical density of approximately 1.00 in the control (B₀) wells containing zero hormone. Once the plates had developed, they were read using a Dynatech MR5000 microplate reader (Dynex Technologies Limited, West Sussex, UK).

2.3.4. General Analysis and Statistical Methods

Statistical analysis and production of graphical representations of the data were performed using the software packages SAS, version 9.3 (SAS Institute Inc., 2002-2010) and Microsoft Office Excel (Microsoft Corporation, 2007b).

Chapter Three.

Investigation of Environmental Factors Affecting the Race Performance of British Greyhounds

3. Investigation of Environmental Factors Affecting the Race Performance of British Greyhounds

3.1. Introduction

Races follow the same format on all GBGB-licensed race tracks in the UK. Greyhounds are raced in an anticlockwise direction around an elliptical track with a sand surface material. Races consist of mixed male and female dogs, with a maximum of six dogs per race. For the majority of races, the starting trap number is allocated by the Racing Manager in a non-random manner, usually taking into consideration the preference of the Greyhound for running close to the hare rail, middle or outer perimeter of the track. Greyhound stadiums vary widely in terms of track design, circumference, radius of the bends, angles of inclination, track surface conditions and preparation methods.

Frequently used measures of Greyhound performance include the quantitative traits of racing speed, race time over a specific distance and rank finish position in a race. Such characteristics are controlled by many different genes at different loci, and are also affected by non-genetic (environmental) influences such as nutrition, management, training, climate, geographic location or exposure to disease (Simm, 1998).

3.1.1. Environmental Factors Affecting the Race Performance of Greyhounds

Environmental factors of sex of the dog, fitness level, grade of race, speed, race distance, track design, degree of banking and radius of the bends, track surface material and conditions, track maintenance and the weather have been reported to affect the rate and type of Greyhound injuries that occur during racing (Cook, 1998; Davis, 1973; Gannon, 1972; Hickman, 1975; Sicard *et al.*, 1999; Prole, 1976; Vaughan, 1969). Such factors are also likely to influence the running times and performance of racing Greyhounds generally (Cook, 1998). Studies of Irish racing Greyhounds and of racing Whippets found that the non-genetic (environmental) factors of sex of the dog, race stadium, individual race effect, season of the race and racing age had a statistically significant effect on several race performance traits (Meissen, 1997; Ryan, 1976; Taubert *et al.*, 2007).

Greyhound daily food intake and the nutrient composition of diets have been reported to affect the racing performance of Greyhounds in the USA (Gannon, 1987; Grandjean and Paragon, 1992; Hill, 1998; Hill *et al.*, 2000, 2001, 2005; Kesl and Engen, 1998; Marshall *et al.*, 2002; Scott *et al.*, 2001; Toll *et al.*, 1992). The majority of racing Greyhounds are fed a commercial extruded diet containing 20% to 28% Metabolisable Energy (ME) protein and 25% to 45% ME fat. Additional meat (often beef) is usually mixed with the extruded diet, which increases dietary protein to greater than 30% ME and increases dietary fat to over 50% ME (Hill *et al.*, 2001; Kohnke, 1998). Toll *et al.* (1992) reported that racing Greyhounds were

slower when dietary fat increased from 31% to 75% of ME. Several authors have suggested that racing Greyhounds should be fed diets with high carbohydrate and low fat content, in order to maximise muscle glycogen content, maximise performance and minimise the extent of lactic acidosis (Gannon, 1987; Grandjean and Paragon, 1992). Hill *et al.* (2000), however, reported that racing Greyhounds ran significantly faster (mean 0.2 s) over a distance of 500 m when dietary fat increased from 25% to 32% of ME, dietary protein increased from 21% to 25% of ME, and carbohydrate decreased from 54% to 43% of ME, although it was uncertain whether the increase in performance was due to changes in dietary protein, fat or carbohydrate. A subsequent study by Hill *et al.* (2001) reported that Greyhounds ran significantly slower (0.18 s) over a distance of 500 m when dietary protein was increased from 24% to 36% of ME and dietary carbohydrate was decreased from 43% to 30% of ME. Hill *et al.* (2001) suggest that excess quantities of dietary protein and fat, or inadequate quantities of dietary fat and carbohydrate, may both have a negative effect on race performance in Greyhounds. Many Greyhound trainers supplement the diet with additional vitamins and minerals. High daily doses of Vitamin E (1,000 international units) (Hill *et al.*, 2001) or Vitamin C (1 g) (Marshall *et al.*, 2002), however, have been reported to slow racing Greyhounds.

An adult trained Greyhound in racing condition has a typical body condition score of approximately 3.5 on a 9-point scale (Hill *et al.*, 2005). It is common practice for many Greyhound trainers to mildly restrict the food intake of racing dogs, due to the traditional belief that Greyhounds run faster when they are leaner (Hill *et al.*,

2005). Hill *et al.* (2005) reported that Greyhounds receiving a restricted food intake (85% of free choice daily food intake) ran 0.19 m/s faster over a 500 m sprint distance, weighed 6% less and had a lower body condition score (3.5 compared to 3.75 on a 9-point scale) than Greyhounds given unrestricted access to food.

Studies investigating environmental influences on race performance of British Greyhounds have, to date, focussed on the investigation of hormonal factors affecting performance (Payne, 2013a,b). In a study of performance changes in dioestrus of 4,453 Greyhound bitches from July 1996 to May 2012, Payne (2013a) reported variations in female Greyhound performance according to the stage of the canine oestrus cycle. The study identified a decline in performance ranging from 0.031 s to 0.733 s (over a 450 m race distance) between 41 and 56 days post-oestrus, and a gradual return to baseline (anoestral) performance level by approximately 80 to 100 days post-oestrus. In a subsequent study, Payne (2013b) investigated the effect of ovariohysterectomy on female Greyhound racing performance, and reported no significant differences in performance between entire and neutered Greyhound bitches. The influence of non-hormonal environmental factors on the racing performance of British Greyhounds have not previously been reported.

3.1.2. Environmental Factors Affecting the Race Performance of Thoroughbred Horses

In Thoroughbred racehorses, environmental factors of sex, age, year, season, class of race, distance of race, handicap weight, type of track, track condition, post-position, trainer effect, jockey effect and individual race effect have been reported to influence race performance (Bugislaus *et al.*, 2005; Ekiz *et al.*, 2005b; Ekiz and Kocak, 2007; Mota *et al.*, 1998, 2005; Oki *et al.*, 1994, 1995; Svobodova *et al.*, 2005; Thiruvankadan *et al.*, 2009a). Studies of racehorse stadiums have found that track surface conditions, such as substrate type, bulk density and moisture content, can influence performance by affecting racing speed (Field *et al.*, 1993; Zebarth and Sheard, 1985). There is little published research investigating the relationship between Greyhound race performance and track conditions, however it is likely that variations in track surface characteristics will have a similar affect on performance in racing Greyhounds (Cook, 1998). Climatic conditions such as temperature, precipitation and wind may also affect Greyhound race performance.

3.2. Aims

The aims of the study were to establish a comprehensive performance and pedigree database for GBGB-licensed racing Greyhounds in the UK, in order to investigate the effects of a number of environmental (non-genetic) factors on variation in British Greyhound race performance. The results of the study will

determine the environmental factors to consider in later genetic analysis of the data.

3.3. Materials and Methods

3.3.1. Data Collection and Cleaning for Quality Control

Historical race performance and phenotype data were obtained for each Greyhound competing in all races that took place at GBGB-licensed Greyhound tracks during a five-year period from 1st January 2008 to 31st December 2012, using the publically available online resource Greyhound-Data (2014). In total, 1,882,658 race performance records for 52,826 individual Greyhounds were obtained and collated into a Microsoft Access database (Microsoft Corporation, 2007) as detailed in Chapter 2.1.1. Data cleaning and quality control steps were performed as detailed in Chapter 2.1.2. The final data set for analysis contained a total of 1,711,489 individual race performance records for 50,452 individual Greyhounds (Appendix 1).

3.3.2. Analysis of Non-Genetic (Environmental) Factors Affecting the Racing Performance of British Greyhounds

Greyhounds are traditionally selected for breeding based on their own or their relative's phenotypic performance in terms of racing speed, race time over a specific distance and/or rank finish position in a race (Taubert *et al.*, 2007).

Consequently, these three measures of racing performance were chosen for investigation in the present study.

The most common Greyhound race distance in the UK is the 480 m flat race (Greyhound Board of Great Britain, 2009). Analysis was initially performed on a smaller subset of the data, consisting of performance results for a race distance of 480 m only ($n = 481,882$ race performance results). This was to enable analysis of the industry-important performance trait 'Race Time', which requires performance results over a fixed racing distance. Additionally, this enabled comparisons to be made with the previously reported Irish Greyhound studies which analysed performance results for 480 m races only (Ryan, 1975; Taubert *et al.*, 2007). Separate analysis was carried out on the complete data set ($n = 1,711,489$ race performance results), to include all performance results over all race distances.

In this and all subsequent analyses, the performance trait 'Rank' refers to the adjusted rank value, *AdjRank*, to correct the Rank result according to the number of Greyhounds in the race as detailed in Chapter 2.1.3.5. The performance trait 'Speed' refers to the average speed (m/s) of the dog during the race, and the performance trait 'Race Time' refers to the time taken for a Greyhound to complete a race of 480 m distance. Descriptions and classifications of the performance data and environmental variables are detailed in Chapter 2.1.

Univariable analyses were performed by analysis of variance (ANOVA) in SAS using the PROC ANOVA function, in order to determine the independent variables to

include in each of the separate general linear models for the dependent variables Race Time, Speed and Rank. ANOVA involves partitioning the observed variation in a particular variable into components of variation between and within different groups or classes.

The model building process was performed at the Swedish University of Agricultural Sciences (SLU) (Uppsala, Sweden) (E. Strandberg). The GLM procedure in SAS (SAS Institute Inc., 2002-2010) uses the method of least squares to fit general linear models. Using the performance data for a race distance of 480 m only (n = 481,882 race performance results), the PROC GLM function in SAS was used to fit a general linear model for the dependent variable Race Time over 480 m, with independent variables: *Sex* (males = 1, females = 2), *BirthYrInd* (2001 - 2011), *Ndogs* (4 - 6), *Trap* (1 - 6), *StadiumID* (1 - 26), *MYStad* (1,509 levels) and a cubic regression to analyse the influence of the covariate *RacingAge* (15 - 98 months). Similarly, the PROC GLM function was used to fit two separate general linear models for the dependent variables Speed over all distances and Rank over all distances, respectively. Both of these models contained the independent variables: *Sex* (males = 1, females = 2), *BirthYrInd* (2001 - 2011), *RaceType* (1 -4), *Ndogs* (4 - 6), *Trap* (1 - 6), *StadiumID* (1 - 26), *MYStad* (1,509 levels) and cubic regressions to analyse the influence of the covariates *RacingAge* (15 - 98 months) and *RaceDist* (220 m to 1,080 m).

Due to the large data set produced in the present study, computational limits of the SAS software prevented inclusion of the independent variable *RaceID* (319,219

levels) to investigate the environmental influence of individual race event on racing performance. Consequently, the effect of month-year-stadium combination, *MYStad* (1,509 levels), was examined to investigate the combined environmental effects of stadium and seasonal climatic conditions on Greyhound race performance, as detailed in Chapter 2.1.3.2.

The PROC GLM function in SAS was used to perform three separate analyses on each of the three models, in order to investigate the influence of specific environmental (non-genetic) factors on the performance traits Race Time over 480 m, and Speed and Rank over all distances, in British Greyhounds.

3.4. Results

3.4.1. Summary Statistics

The distribution of race performance records per dog during a five-year period from 1st January 2008 to 31st December 2012 is displayed in Figure 3.1.

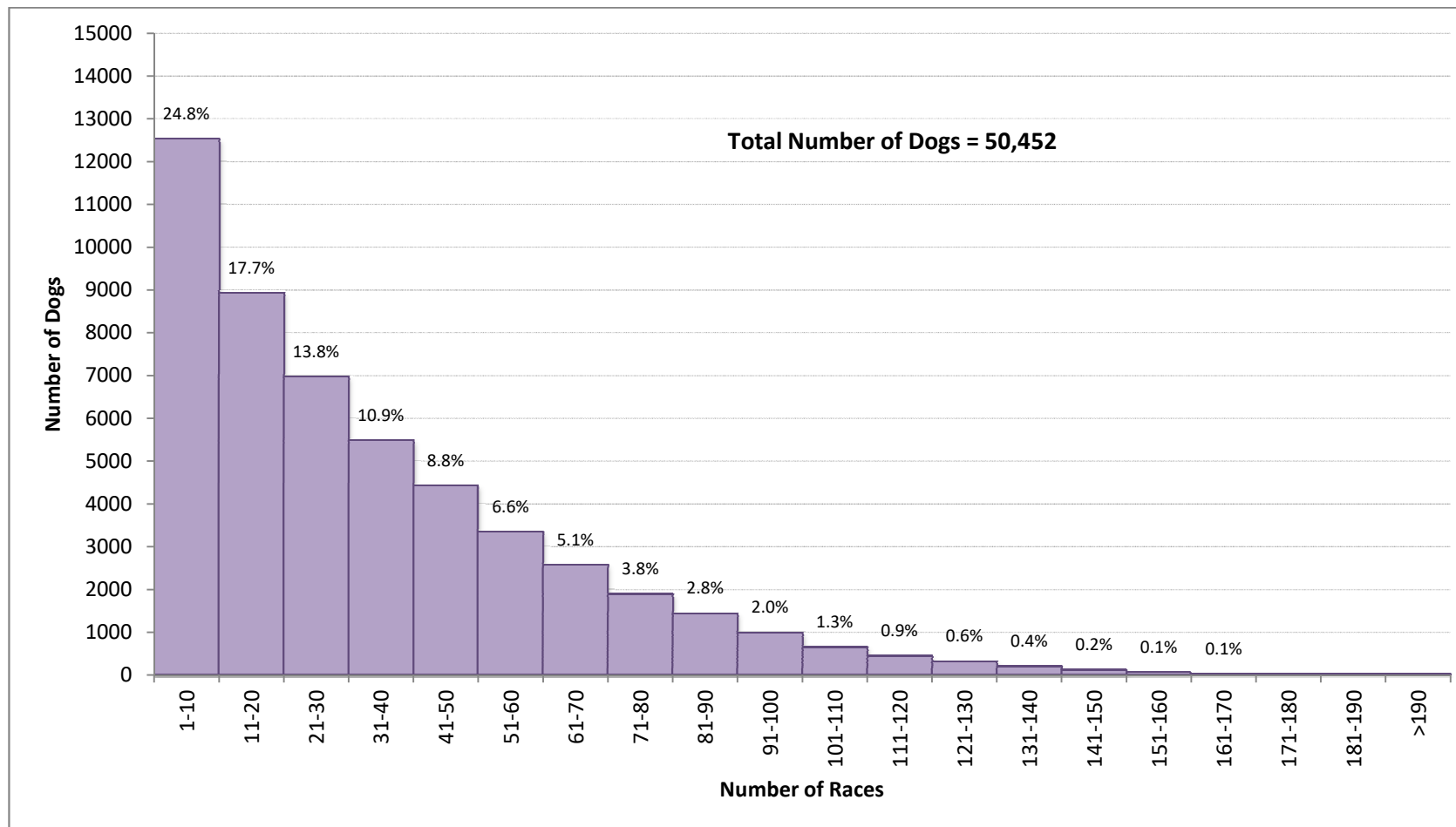


Figure 3.1. The distribution of number of race performance records per dog (n = 50,452) during a five-year period from 2008-2012, in British Greyhounds.

The mean, standard deviation (SD), minimum and maximum values for the performance traits Race Time, Speed and Rank are displayed in Table 3.1 and Table 3.2 for performance results over a race distance of 480 m and for results over all race distances, respectively.

Performance Trait	N	Mean	SD	Min	Max
Race Time (s)	481882	29.86	0.59	27.39	41.53
Speed (m/s)	481882	16.08	0.32	11.55	17.52
Rank	481882	3.47	1.70	1.00	6.00

Table 3.1. Number of race performance results (N), mean, standard deviation (SD), minimum (Min) and maximum (Max) values for the performance traits Race Time (s), Speed (m/s) and Rank (1 - 6) over a race distance of 480 m in British racing Greyhounds.

Performance Trait	N	Mean	SD	Min	Max
Race Time (s)	1711489	29.36	3.96	13.28	71.56
Speed (m/s)	1711489	16.01	0.41	10.99	17.93
Rank	1711489	3.47	1.70	1.00	6.00

Table 3.2. Number of race performance results (N), mean, standard deviation (SD), minimum (Min) and maximum (Max) values for the performance traits Race Time (s), Speed (m/s) and Rank (1 - 6) in British racing Greyhounds over all race distances (220 m to 1,080 m).

The mean, SD, minimum and maximum values for the performance traits Race Time, Speed and Rank, are displayed in Table 3.3 for performance results within *RaceType* 1 (race distances ≤ 305 m), *RaceType* 2 (race distances > 305 m and < 539 m), *RaceType* 3 (race distances ≥ 540 m and ≤ 700 m) and *RaceType* 4 (race distances > 700 m).

Performance Trait	Race Type	N	Mean	SD	Min	Max
Race Time (s)	1	38765	16.76	1.01	13.28	20.37
	2	1534968	28.83	1.96	22.76	41.53
	3	128789	38.01	2.78	33.07	49.61
	4	8967	49.3	4.22	42.33	71.56
Speed (m/s)	1	38765	16.3	0.55	12.78	17.93
	2	1534968	16.03	0.4	10.99	17.58
	3	128789	15.69	0.37	13.34	17.04
	4	8967	15.44	0.41	14.19	17
Rank	1	38765	3.47	1.71	1	6
	2	1534968	3.47	1.7	1	6
	3	128789	3.47	1.7	1	6
	4	8967	3.45	1.7	1	6

Table 3.3. Number of race performance results (N), mean, standard deviation (SD), minimum (Min) and maximum (Max) values for the performance traits Race Time (s), Speed (m/s) and Rank (1 - 6) in British racing Greyhounds according to Race Type (1 - 4). Where: Race Type 1 = race distances ≤ 305 m; Race Type 2 = race distances > 305 m and < 539 m; Race Type 3 = race distances ≥ 540 m and ≤ 700 m; Race Type 4 = race distances > 700 m.

3.4.2. Influence of Non-Genetic (Environmental) Factors on the Race Performance of British Greyhounds

Table 3.4 displays the results of the univariable analyses of the influence of various environmental variables on each measure of Greyhound performance.

	PERFORMANCE MEASURE		
ENVIRONMENTAL VARIABLE	Race Time (480 m distance)	Speed (all distances)	Rank (all distances)
Sex	F Value 6487.94 Pr > F = < 0.0001	F Value: 24497.4 Pr > F = < 0.0001	F Value: 332.23 Pr > F = < 0.0001
BirthYrInd	F Value: 833.83 Pr > F = < 0.0001	F Value: 1585.92 Pr > F = < 0.0001	F Value: 92.86 Pr > F = < 0.0001
RaceType		F Value: 42260.4 Pr > F = < 0.0001	F Value: 0.63 Pr > F = 0.5969
Ndogs	F Value: 316.02 Pr > F = < 0.0001	F Value: 13.11 Pr > F = < 0.0001	F Value: 0.23 Pr > F = 0.7906
Trap	F Value: 9.02 Pr > F = < 0.0001	F Value: 41.06 Pr > F = < 0.0001	F Value: 546.71 Pr > F = < 0.0001
Stadium	F Value: 12847.4 Pr > F = < 0.0001	F Value: 69922.1 Pr > F = < 0.0001	F Value: 1.35 Pr > F = 0.1143
MYStad	F Value: 420.03 Pr > F = < 0.0001	F Value: 1446.99 Pr > F = < 0.0001	F Value: 0.06 Pr > F = 1.0000
RacingAge	F Value: 93.56 Pr > F = < 0.0001	F Value: 212.74 Pr > F = < 0.0001	F Value: 33.56 Pr > F = < 0.0001
RaceDist		F Value: 11810.1 Pr > F = < 0.0001	F Value: 0.20 Pr > F = 1.0000

Table 3.4. Results of the univariable analyses of the influence of various environmental (non-genetic) variables on the performance measures Race Time (over a 480 m distance), Speed and Rank (over all distances). F Values and *p* - values are given. A grey coloured block indicates that the variable was not tested.

In analysis of the multivariable models, the non-genetic (environmental) factors of *Sex* ($p < 0.0001$), *BirthYr* ($p < 0.0001$), *Ndogs* ($p < 0.0001$), *Trap* ($p < 0.0001$), *Stadium* ($p < 0.0001$), *MYStad* ($p < 0.0001$) as well as the covariate *RacingAge* ($p < 0.0001$), *RacingAge*² ($p < 0.0001$) and *RacingAge*³ ($p < 0.0001$), were found to be significantly associated with the performance trait Race Time over a 480 m race distance.

For the complete data set including races over all distances, the environmental factors of *Sex* ($p < 0.0001$), *BirthYr* ($p < 0.0001$), *RaceType* ($p < 0.0001$), *Ndogs* ($p < 0.0001$), *Trap* ($p < 0.0001$), *Stadium* ($p < 0.0001$) and *MYStad* ($p < 0.0001$), as well as the covariates *RacingAge* ($p < 0.0001$), *RacingAge*² ($p < 0.0001$), *RacingAge*³ ($p < 0.0001$), *RaceDist* ($p < 0.0001$), *RaceDist*² ($p < 0.0001$) and *RaceDist*³ ($p < 0.0001$), were found to be significantly associated with the performance trait Speed.

The environmental factors of *Sex* ($p < 0.0001$), *BirthYr* ($p < 0.0001$), *Trap* ($p < 0.0001$), *MYStad* ($p < 0.0001$) and the covariate *RacingAge* ($p < 0.0005$) were significantly associated with the performance trait Rank. Environmental factors of *RaceType*, *Stadium* and the covariates *RacingAge*², *RacingAge*³, *RaceDist*, *RaceDist*² and *RaceDist*³ had no statistically significant effect ($p > 0.05$) on Rank.

The direction of significance for each factor is described in Chapters 3.4.2.1 to 3.4.2.9.

The results of the multivariable model analyses are summarised in Table 3.5.

ENVIRONMENTAL VARIABLE	PERFORMANCE MEASURE		
	Race Time (480 m distance)	Speed (all race distances)	Rank (all race distances)
Sex	**	**	**
BirthYrInd	**	**	**
RaceType		**	n.s.
Ndogs	**	**	
Trap	**	**	**
Stadium	**	**	
MYStad	**	**	**
RacingAge	**	**	*
RacingAge ²	**	**	n.s.
RacingAge ³	**	**	n.s.
RaceDist		**	n.s.
RaceDist ²		**	n.s.
RaceDist ³		**	n.s.

Table 3.5. Summary of all the environmental (non-genetic) variables tested and the statistical significance of results on race performance measures in British Greyhounds. Where: n.s. = not significant $p > 0.05$; * = significant $p < 0.0005$; ** = significant $p < 0.0001$. A grey coloured block indicates that the effect was not tested.

3.4.2.1. *Sex of the Greyhound (Sex)*

The ratio of sexes in the data set was almost 1:1, with male dogs comprising 55.52% ($n = 950,184$) and female dogs 44.48% ($n = 761,305$) of the total race results. The sex of the Greyhound was found to be significantly associated with the traits Race Time over 480 m ($p < 0.0001$), Speed ($p < 0.0001$) and Rank ($p < 0.0001$) over all distances. Male Greyhounds had lower race times, faster mean speeds and better rank finish positions than female Greyhounds. The mean monthly racing speeds of male and female Greyhounds during the five-year period from 2008 to 2012 are displayed in Figure 3.2, indicating faster mean speeds for male racing Greyhounds for each month of each year compared to females. In addition, Figure 3.2 demonstrates a phenotypic improvement in the mean racing speed of both male and female British Greyhounds over the five-year study period, from 16.00 m/s to 16.10 m/s and from 15.91 m/s to 15.99 m/s, respectively.

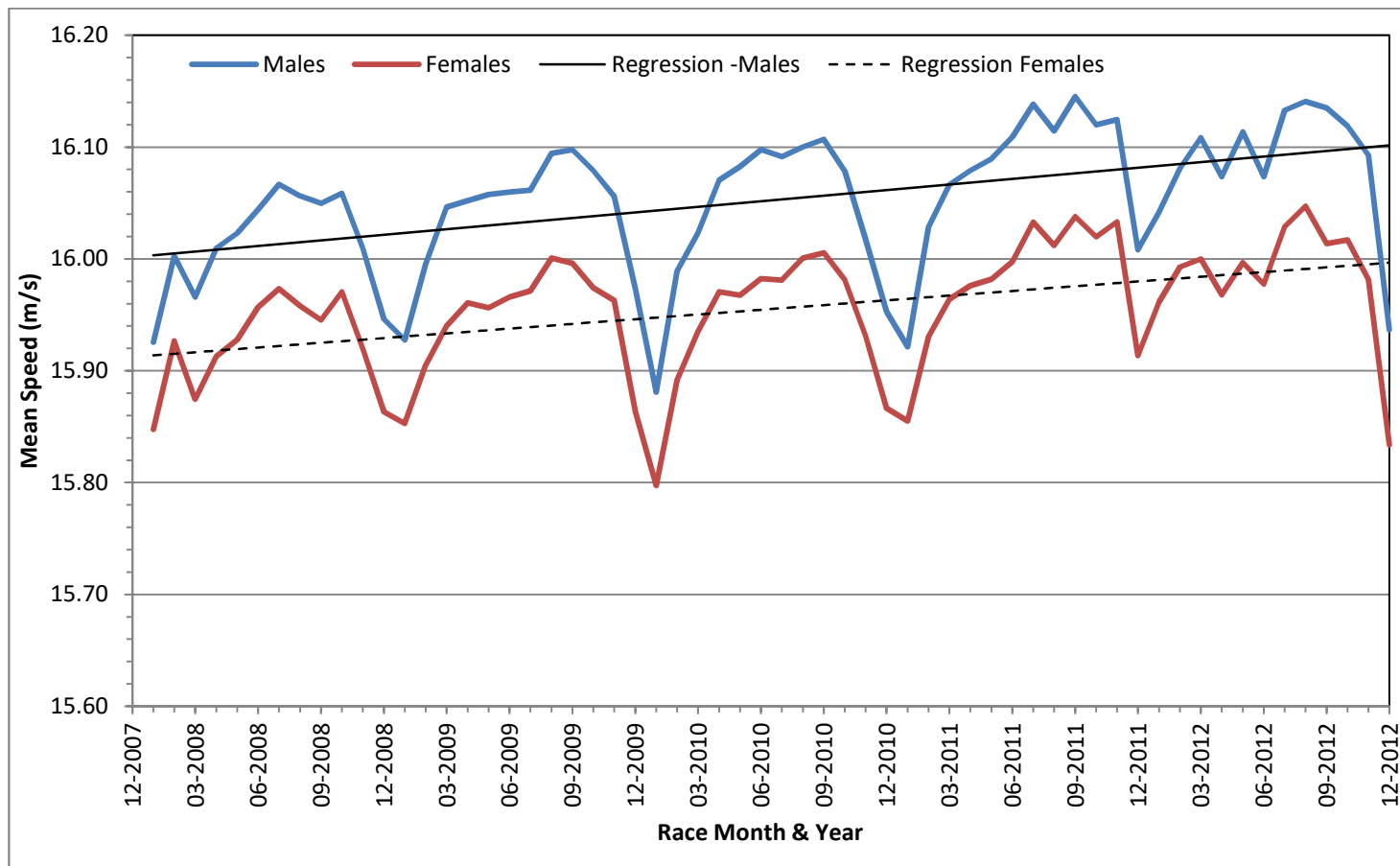


Figure 3.2. The mean monthly racing speeds (m/s) of male (n = 950,184 performance results) and female (n = 761,305 performance results) British racing Greyhounds over all race distances (220 m to 1,080 m) during a five-year period from 2008 to 2012.

3.4.2.2. Greyhound Birth Year (*BirthYrInd*)

The birth year of the Greyhound (2001 - 2011) was found to be significantly associated with the traits Race Time over 480 m ($p < 0.0001$), Speed ($p < 0.0001$) and Rank ($p < 0.0001$) over all distances, with increasing performance as year of birth increases. The mean racing speed according to birth year of the dog is displayed in Figure 3.3, and demonstrates an increase in mean speed with each successive birth year from 2001 to 2010, with a decline in mean speed for Greyhounds born in 2011.

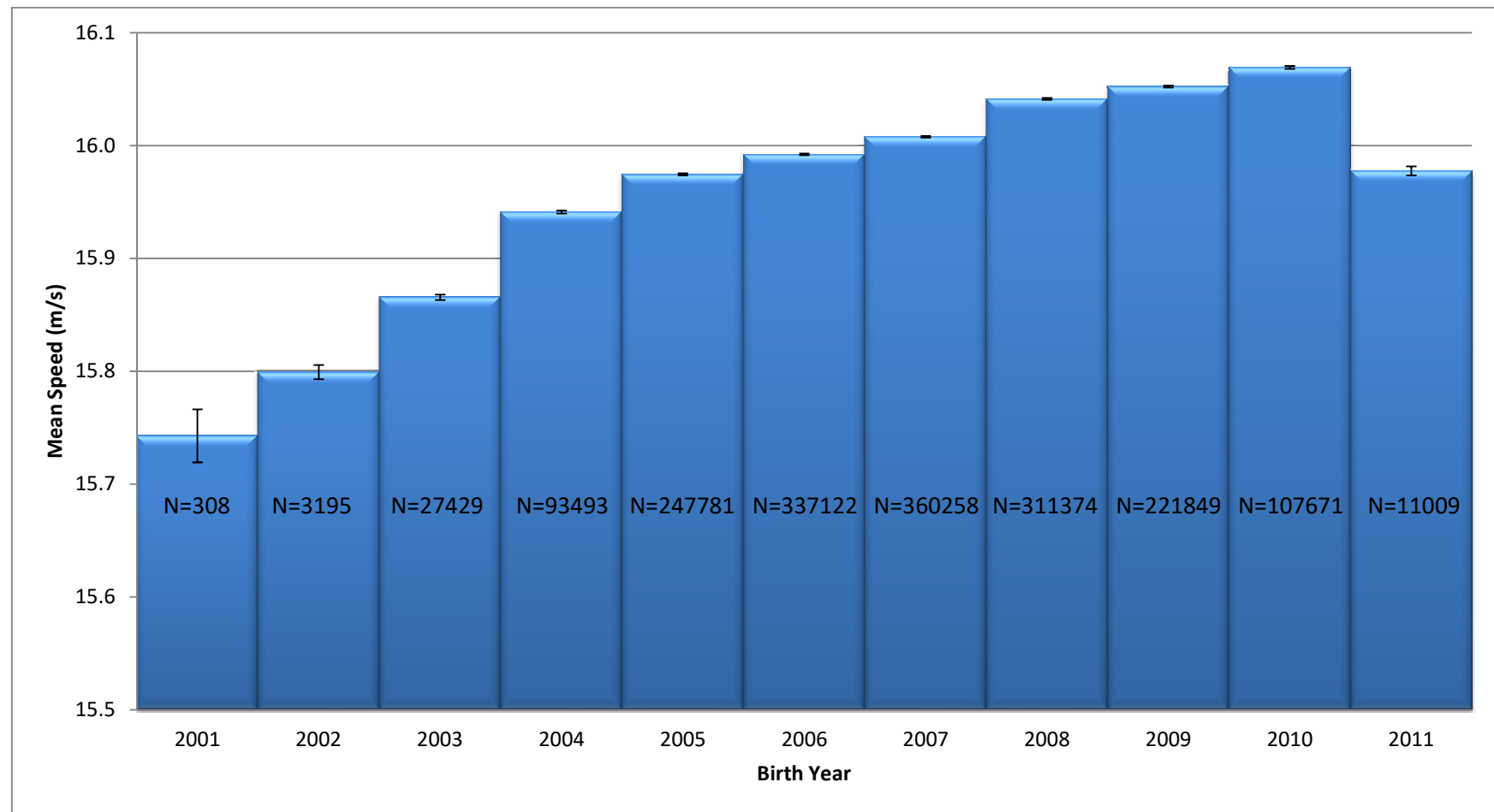


Figure 3.3. The mean racing speed (m/s) of British Greyhounds ($n = 1,711,489$ performance results) over all race distances (220 m to 1,080 m) during a five-year period from 2008 to 2012, according to birth year of the dog. Error bars indicate the standard error of the mean.

3.4.2.3. Greyhound Racing Age (*RacingAge*)

The average age of British Greyhounds at each race start ($n = 1,711,489$ performance results) ranged from 15 months (1.25 years) to 98 months (8.16 years) of age (Figure 3.4), with a mean racing age of 36 months (three years). Racing age was found to be significantly associated with the traits Race Time ($p < 0.0001$) over 480 m, Speed ($p < 0.0001$) and Rank ($p < 0.0005$) over all distances, with a decline in performance with advancing age. The mean racing speed of male and female Greyhounds according to racing age is displayed in Figure 3.5, and indicates a faster mean speed in male Greyhounds of all ages compared to females. Both male and female Greyhounds appear to improve in speed from the start of their racing career to approximately 25 months of age, then decreased gradually in speed at similar rates over the next 45 months.

The mean race time of male and female Greyhounds over a 480 m race distance according to racing age is displayed in Figure 3.6, and indicates a lower mean race time in male Greyhounds of all ages compared to females. Both male and female Greyhounds appear to improve in race time from the start of their racing career to approximately 30 months of age, then declined gradually in race time at similar rates over the next 40 months.

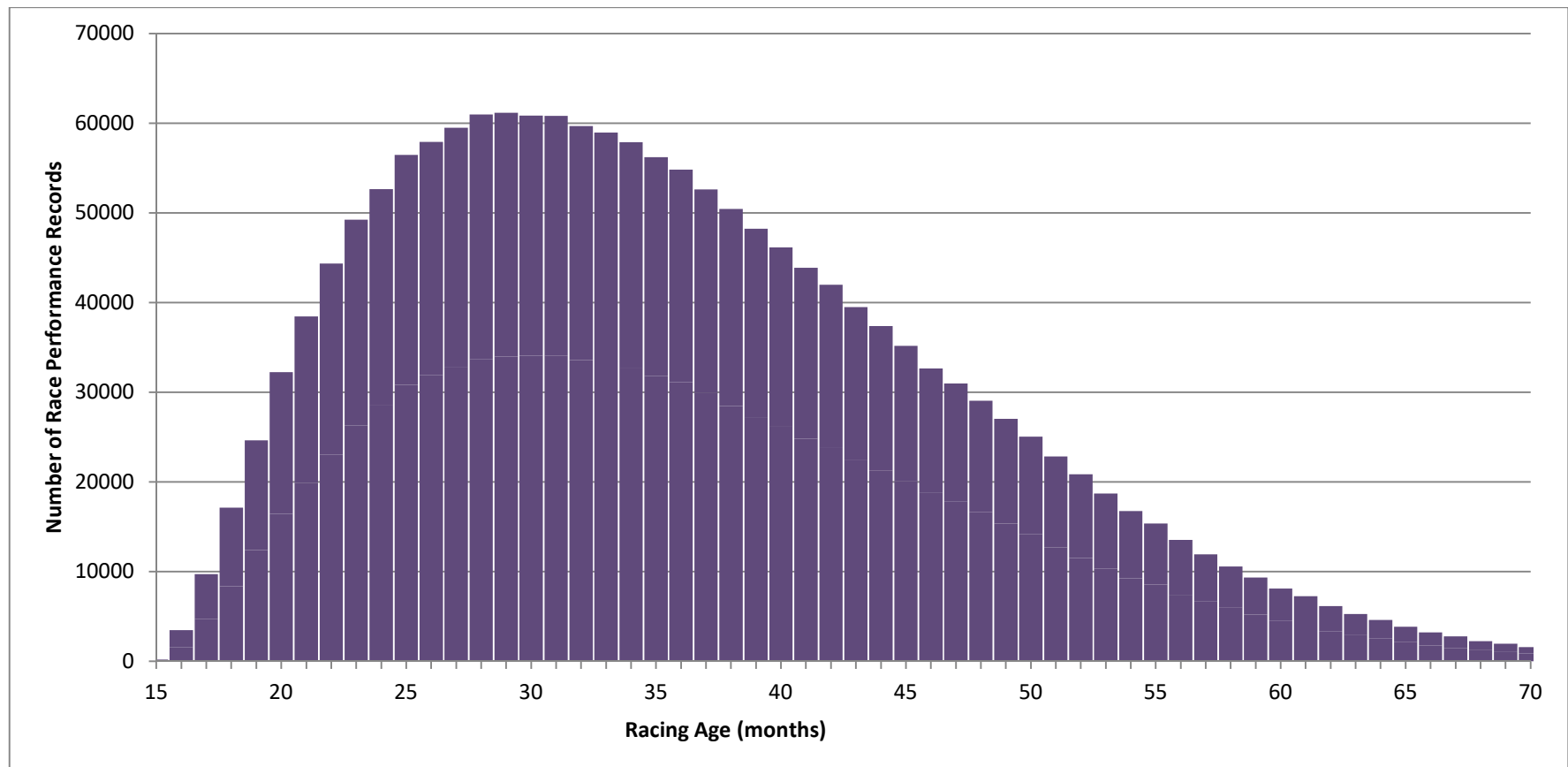


Figure 3.4. The distribution of age of the dog in months at the time of race, per race start, in British Greyhounds (n = 1,711,489 performance results) over a five-year period from 2008 to 2012.

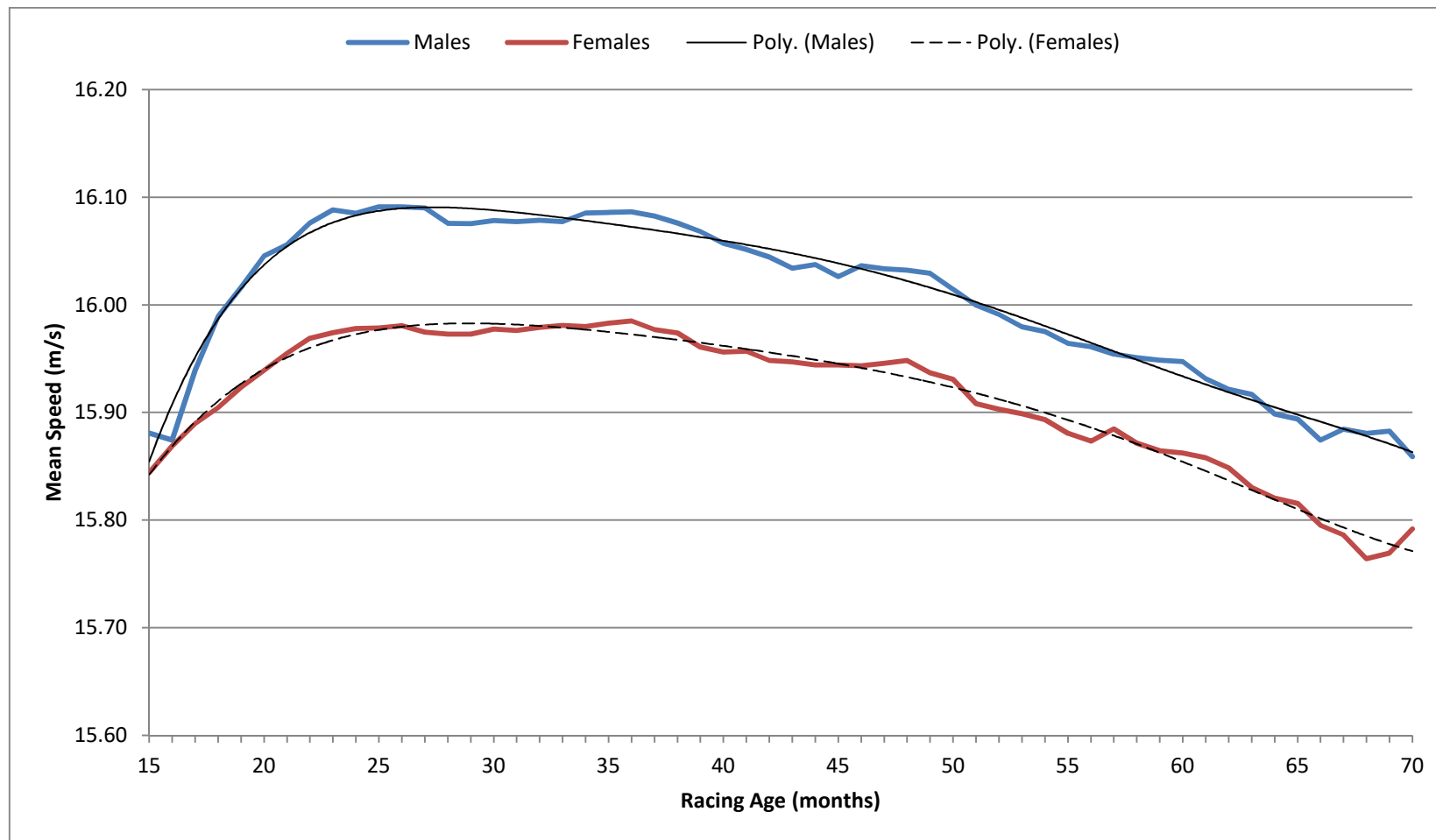


Figure 3.5. Mean race speed (m/s) with respect to racing age (months) in male (n = 950,184 performance results) and female (n = 761,305 performance results) British Greyhounds over all race distances (220 m to 1,080 m) during a five-year period from 2008 to 2012.

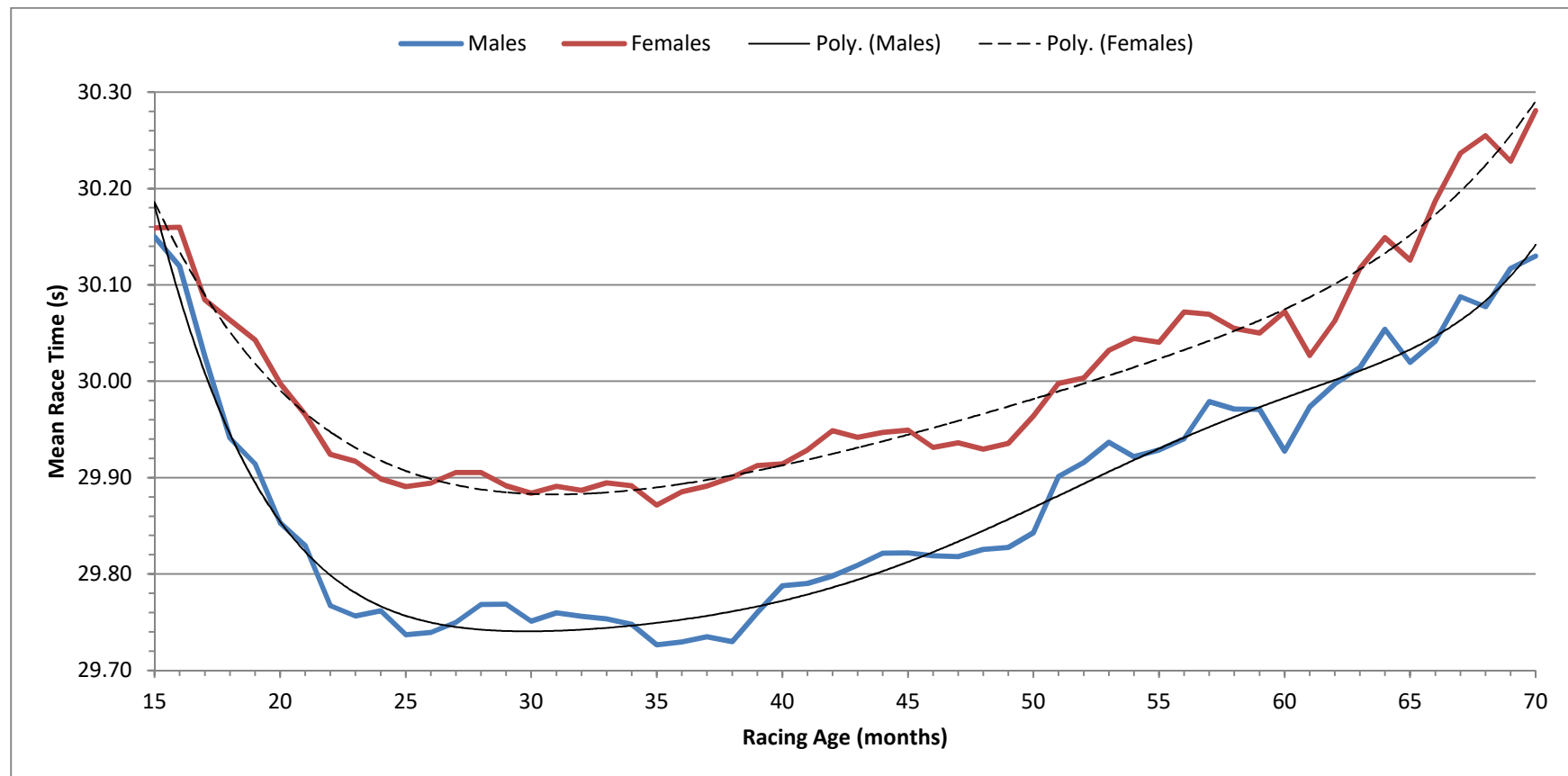


Figure 3.6. Mean race time (s) over a 480 m race distance with respect to racing age (months) in male and female British Greyhounds (n = 481,882 performance results) during a five-year period from 2008 to 2012.

3.4.2.4. *Number of Dogs in the Race (Ndogs)*

In the complete data set, 0.25% ($n = 4,235$) of performance results consisted of four-dog races; 5.14% ($n = 87,927$) consisted of five-dog races; and 94.62% ($n = 1,619,327$) consisted of six-dog races. The number of dogs (4 - 6) in the race was found to be significantly associated with the traits Race Time over 480 m ($p < 0.0001$) and Speed ($p < 0.0001$) over all distances, with greater performance with increased numbers of Greyhounds in the race. The mean racing speed according to number of dogs in the race is displayed in Figure 3.7. Overall, race times were lower and mean speeds faster when there were five dogs competing in a race, where as race times were higher and mean speeds slower when there were four dogs in the race.

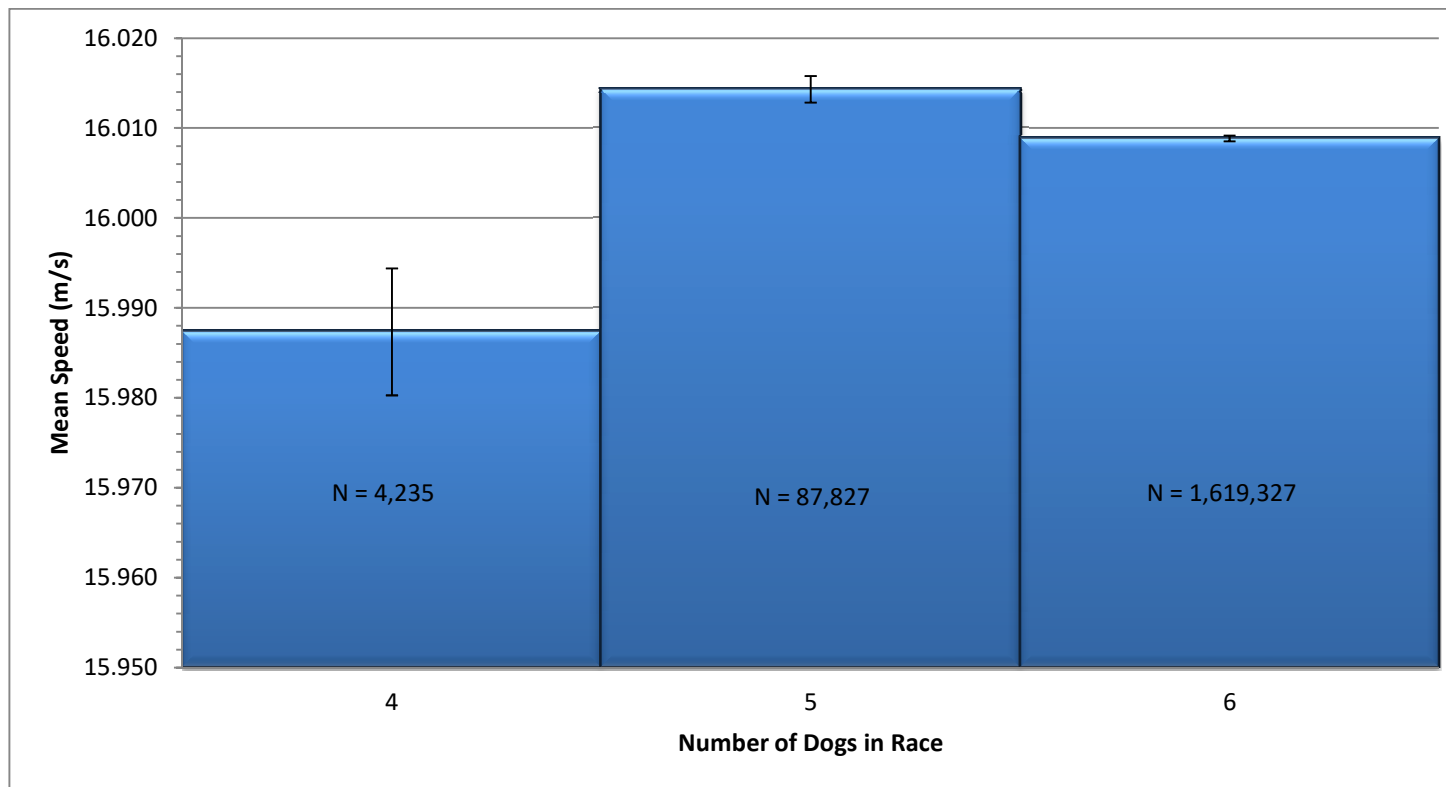


Figure 3.7. The mean racing speed (m/s) of British Greyhounds ($n = 1,711,489$ performance results) over all race distances (220 m to 1,080 m) during a five-year period from 2008 to 2012, according to number of dogs (4-6) in the race. Error bars indicate the standard error of the mean.

3.4.2.5. *Starting Trap Number (Trap)*

The starting trap number (1 - 6) of the Greyhound was found to be significantly associated with the traits Race Time over 480 m ($p < 0.0001$), Speed ($p < 0.0001$) and Rank ($p < 0.0001$) over all distances, with decreasing performance as the trap number increased. The mean racing speed according to trap number is displayed in Figure 3.8, and indicates fastest mean speeds for Greyhounds starting from traps one and six; the traps closest to the inner and outer rail of the track, respectively. Similar results were found for Race Time over 480 m. The mean rank finish position according to trap number is displayed in Figure 3.9, and demonstrates a lower rank value (equivalent to a better finish position, where a rank of one is the winner of the race) for Greyhounds starting from trap number one. Greyhounds starting from trap number five had slowest mean speeds and worst mean rank values.

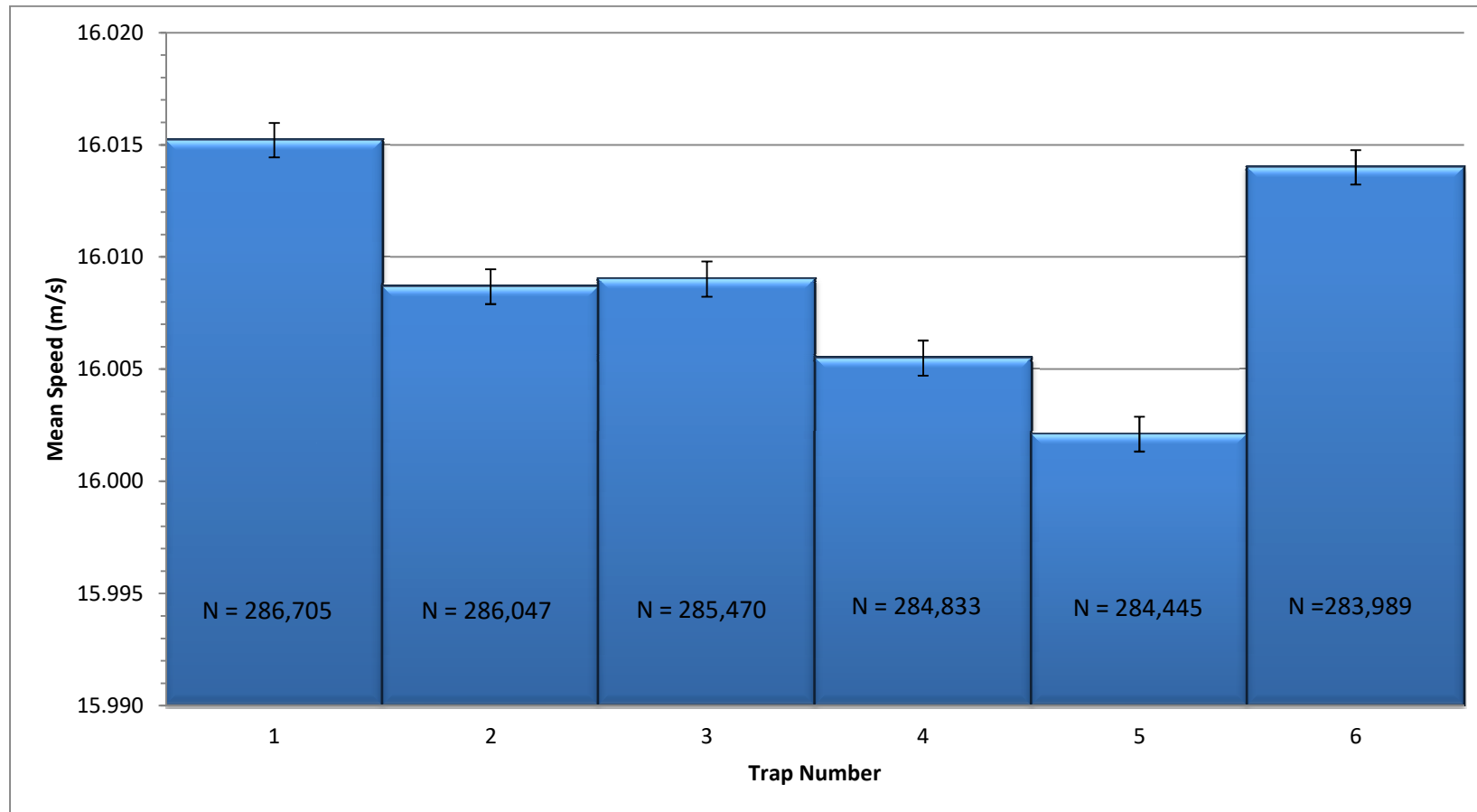


Figure 3.8. The mean racing speed (m/s) of British Greyhounds ($n = 1,711,489$ performance results) over all race distances (220 m to 1,080 m) during a five-year period from 2008 to 2012, according to starting trap number (1-6) in the race. Error bars indicate the standard error of the mean.

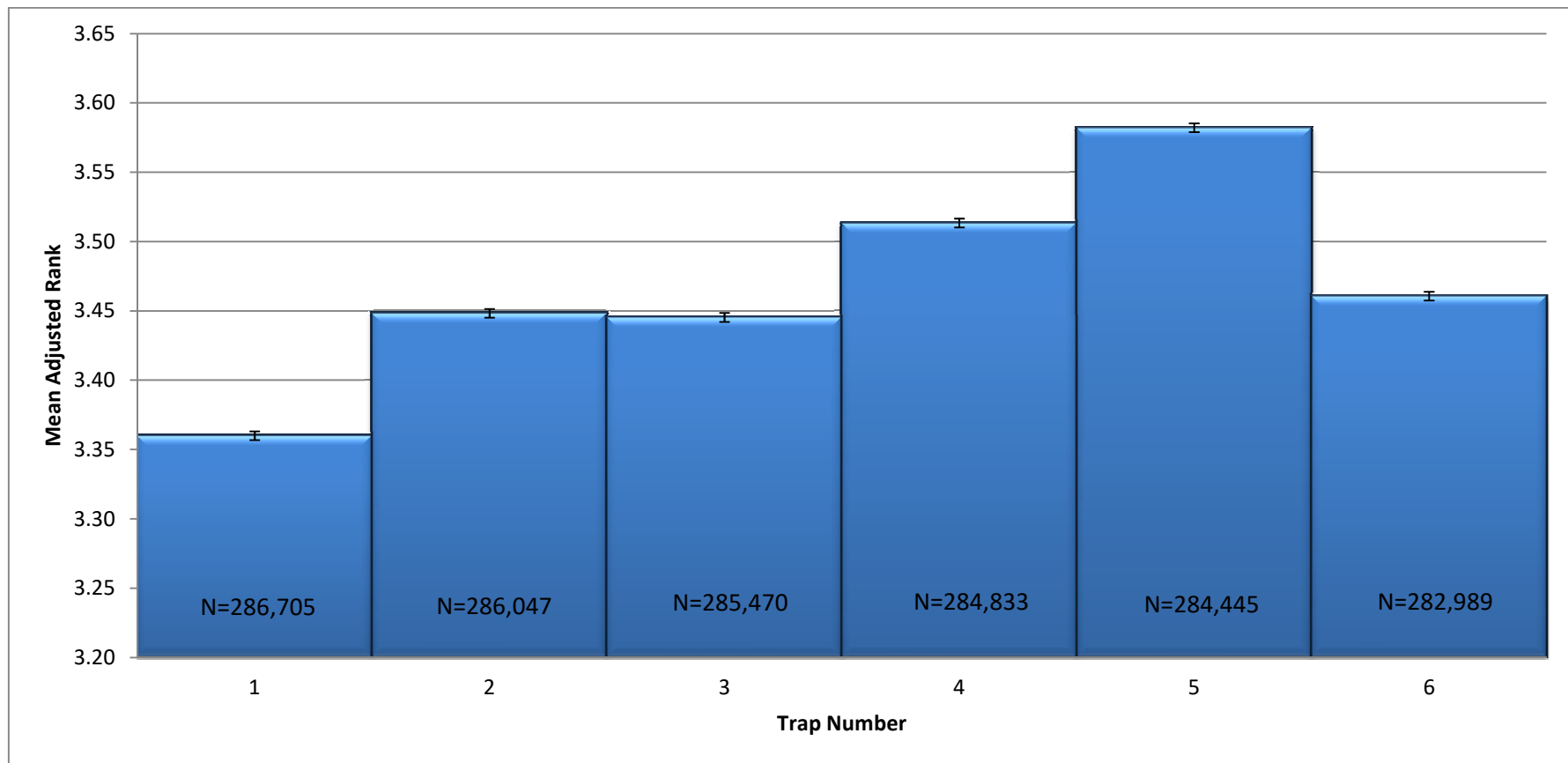


Figure 3.9. The mean rank finish position (1-6) of British racing Greyhounds (n = 1,711,489 performance results) over a five-year period from 2008 to 2012, according to starting trap number (1-6) in the race. A rank of one was awarded to the Greyhound finishing first, and a rank of six awarded to the Greyhound finishing last. Rank results were adjusted prior to analysis to take into account the number of dogs in a race. Error bars indicate the standard error of the mean.

3.4.2.6. *Greyhound Stadium (StadiumID)*

The distribution of race performance results according to Greyhound stadium (1 - 26) is displayed in Figure 3.10. The stadium of the race was found to be significantly associated with the traits Race Time over 480 m ($p < 0.0001$) and Speed ($p < 0.0001$) over all distances, and had no significant effect on Rank ($p > 0.05$). The distribution of mean racing speed according to stadium is displayed in Figure 3.11, indicating considerable variation between the 26 race tracks. Stadium number eight and stadium number six demonstrate fastest and slowest mean speeds of 16.66 m/s and 15.33 m/s, respectively. There were 12 race tracks in the subset of data consisting of 480 m race distances, with fastest and slowest mean race times of 15.76 s and 16.73 s, respectively.

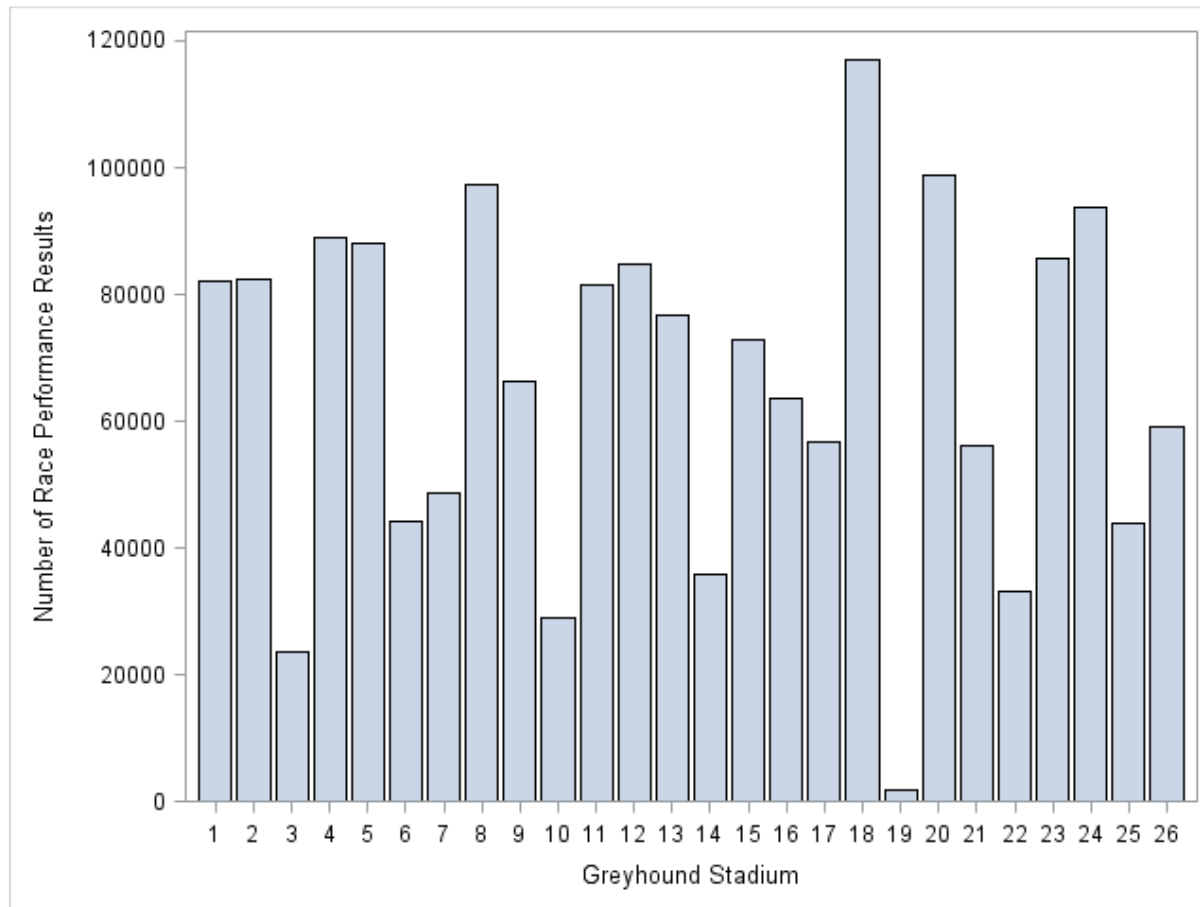


Figure 3.10. The distribution of British Greyhound race performance results (n = 1,711,489) according to Greyhound stadium (1 - 26), over a five-year period from 2008 to 2012.

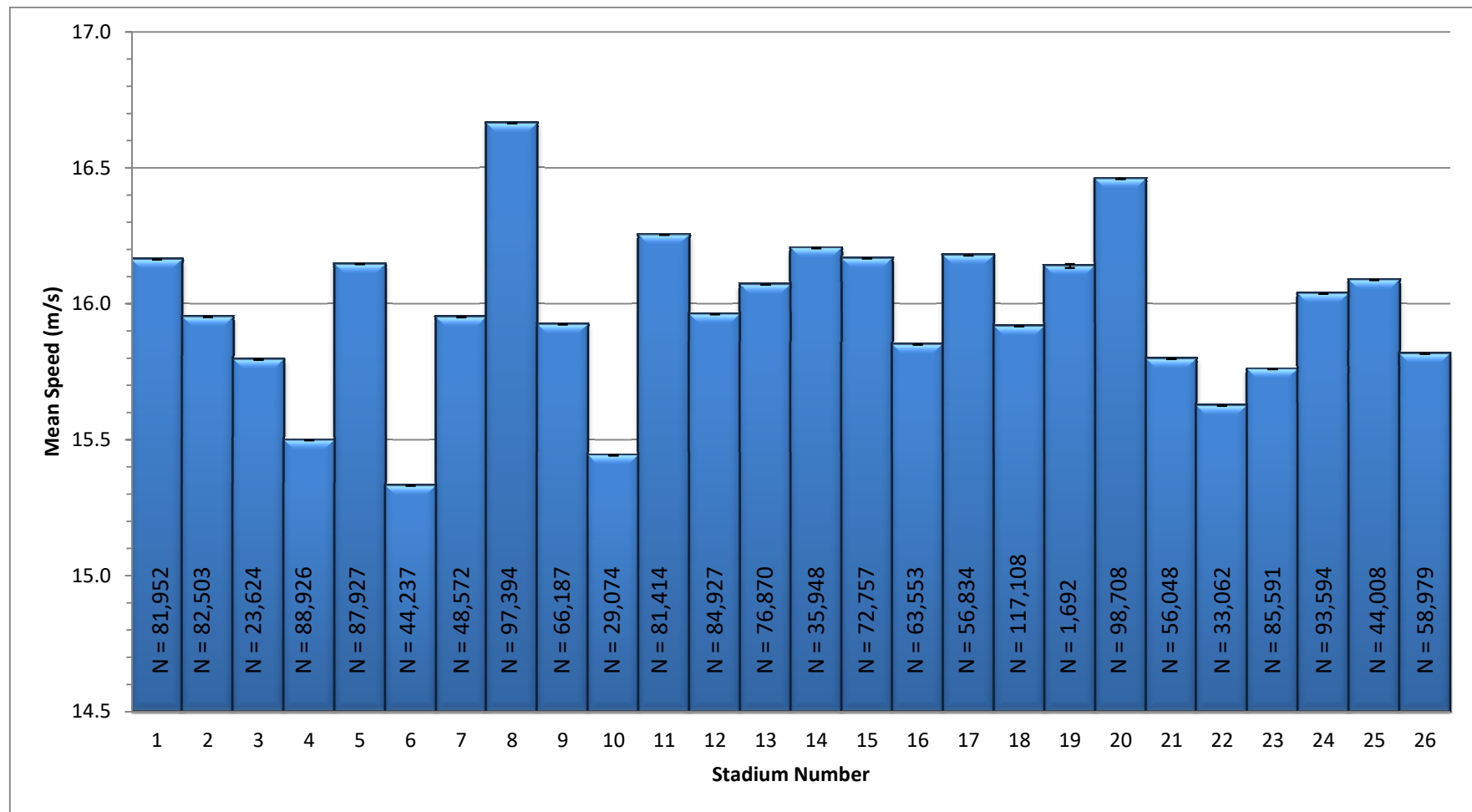


Figure 3.11. The mean racing speed (m/s) of British Greyhounds ($n = 1,711,489$ performance results) over all race distances (220 m to 1,080 m) during a five-year period from 2008 to 2012, according to race stadium (1 - 26). Error bars indicate the standard error of the mean.

3.4.2.7. *Effect of Season and Stadium (MYStad)*

The influence of both season and stadium on racing performance was examined through analysis of the effect of month-year-stadium (*MYStad*) combination of the race. *MYStad* was found to be significantly associated with the traits Race Time ($p < 0.0001$) over 480 m, Speed ($p < 0.0001$) and Rank ($p < 0.0001$) over all distances. The monthly mean race time (s) over a 480 m race distance and monthly mean racing speed (m/s) across all race distances are displayed in Figure 3.12 and Figure 3.13, respectively, indicating seasonal variation in performance associated with monthly mean temperature in the UK. Mean race times and racing speeds were slower in winter months (October to March) when temperatures were lower, and faster in summer months (April to September) when temperatures were higher.

In addition, Figure 3.12 demonstrates an overall phenotypic improvement in the race time of British Greyhounds over a 480 m race distance during the five-year study period, from 29.98 s to 29.74 s. Similarly, Figure 3.13 demonstrates an overall phenotypic improvement in the racing speed of British Greyhounds during the five-year period, from 15.96 m/s to 16.05 m/s.

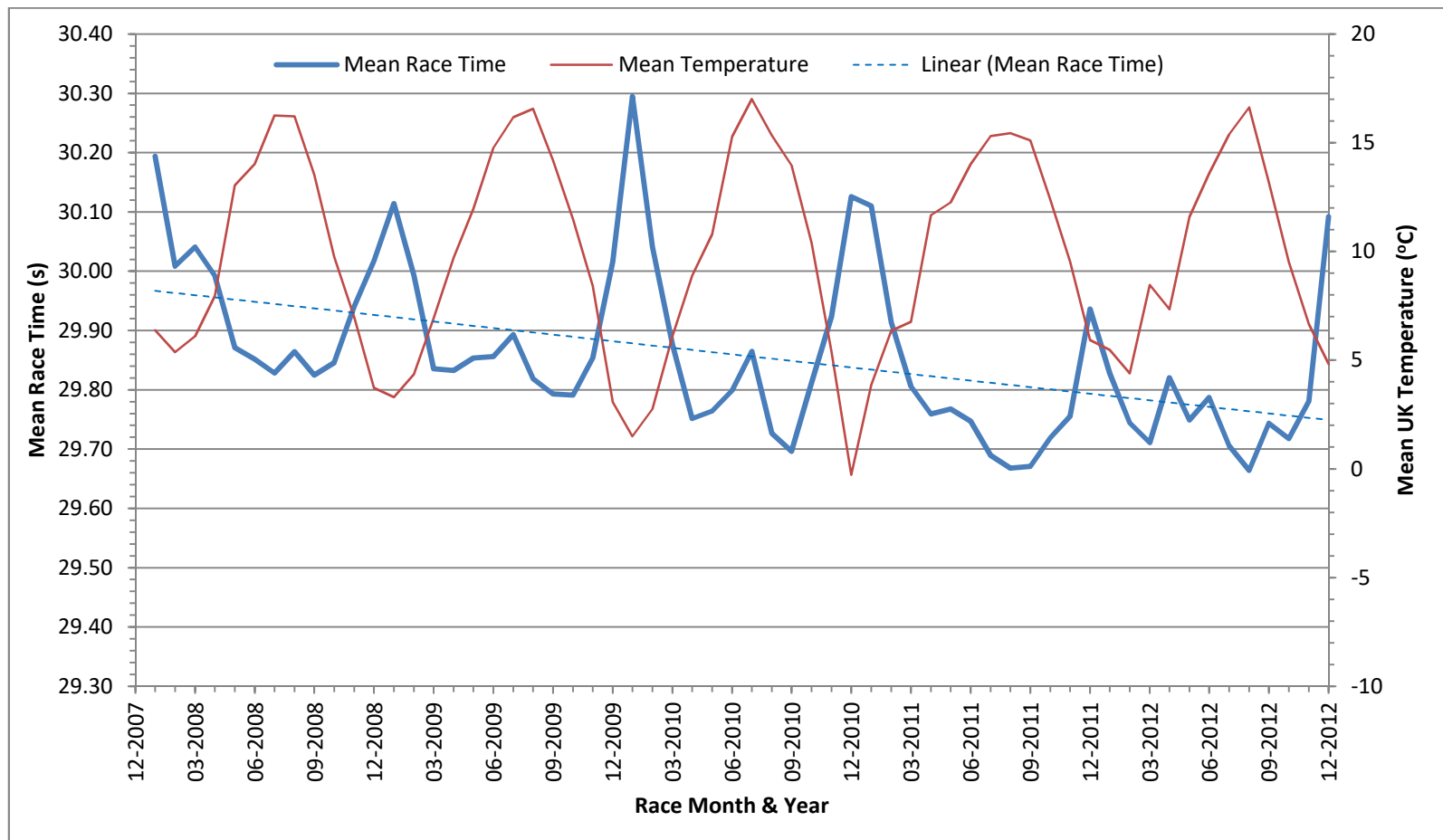


Figure 3.12. The monthly phenotypic mean race times (s) for British racing Greyhounds over a race distance of 480 m (n = 481,882 performance results) during a five-year period from 2008 to 2012, and the monthly mean temperature (degrees Celsius) in the UK. Temperature data obtained from DUKES (2014).

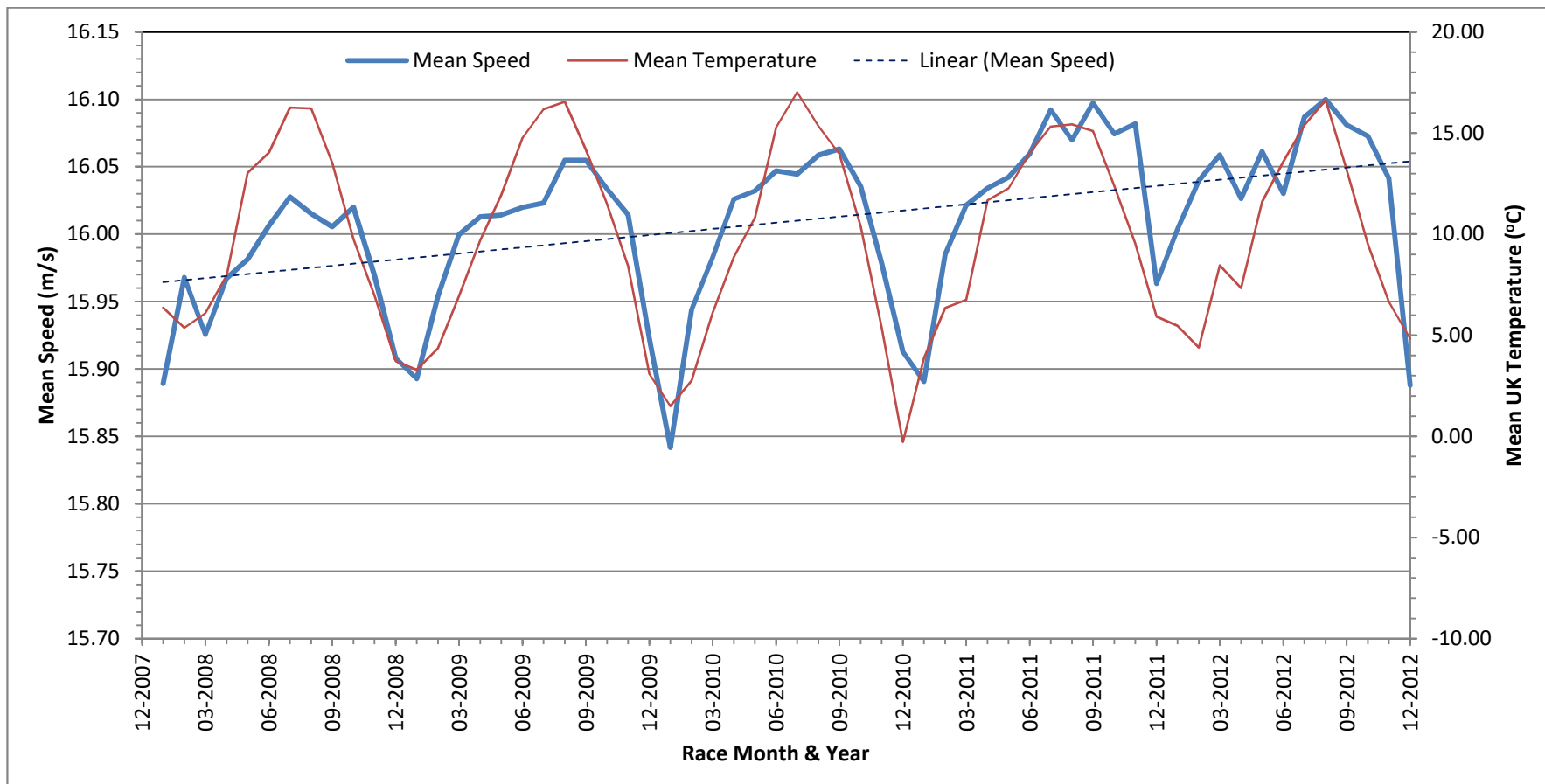


Figure 3.13. The monthly phenotypic mean speeds (m/s) for British racing Greyhounds, based on all performance results ($n = 1,711,489$) across all race distances (220 m to 1,080 m) in a five-year period from 2008 to 2012, and the monthly mean temperature (degrees Celsius) in the UK. Temperature data obtained from DUKES (2014).

3.4.2.8. *Type of Race (RaceType)*

In the complete data set, 2.26% ($n = 38,765$) of performance results were classified as *RaceType* 1; 89.69% ($n = 1,534,968$) as *RaceType* 2; 7.52% ($n = 128,789$) as *RaceType* 3; and 0.52% ($n = 8,967$) as *RaceType* 4. The type of race (1 - 4) was found to be significantly associated with Speed ($p < 0.0001$), and had no significant effect on Rank ($p > 0.05$). The mean racing speed according to race type is displayed in Figure 3.14, and demonstrates a decrease in Greyhound speed with increasing race distance and number of bends to negotiate during the race. The effect of Race Type on the trait Race Time over 480 m was not examined, as all races in this subset of data were of the same distance over a four-bend race, and were therefore classified as *RaceType* 2.

3.4.2.9. *Distance of Race (RaceDist)*

The distance of the race (220 m to 1,080 m) was significantly associated with Speed ($p < 0.0001$), with a decline in mean speed as the race distance increases. The distance of the race had no significant effect on Rank ($p > 0.05$). The mean racing speed (m/s) according to race distance is displayed in Figure 3.15, and indicates a general trend of lower mean speeds for longer race distances. Fastest mean speeds of 17.00 m/s were observed for a race distance of 285 m, and slowest mean speeds of 14.89 m/s were observed for a race distance of 946 m.

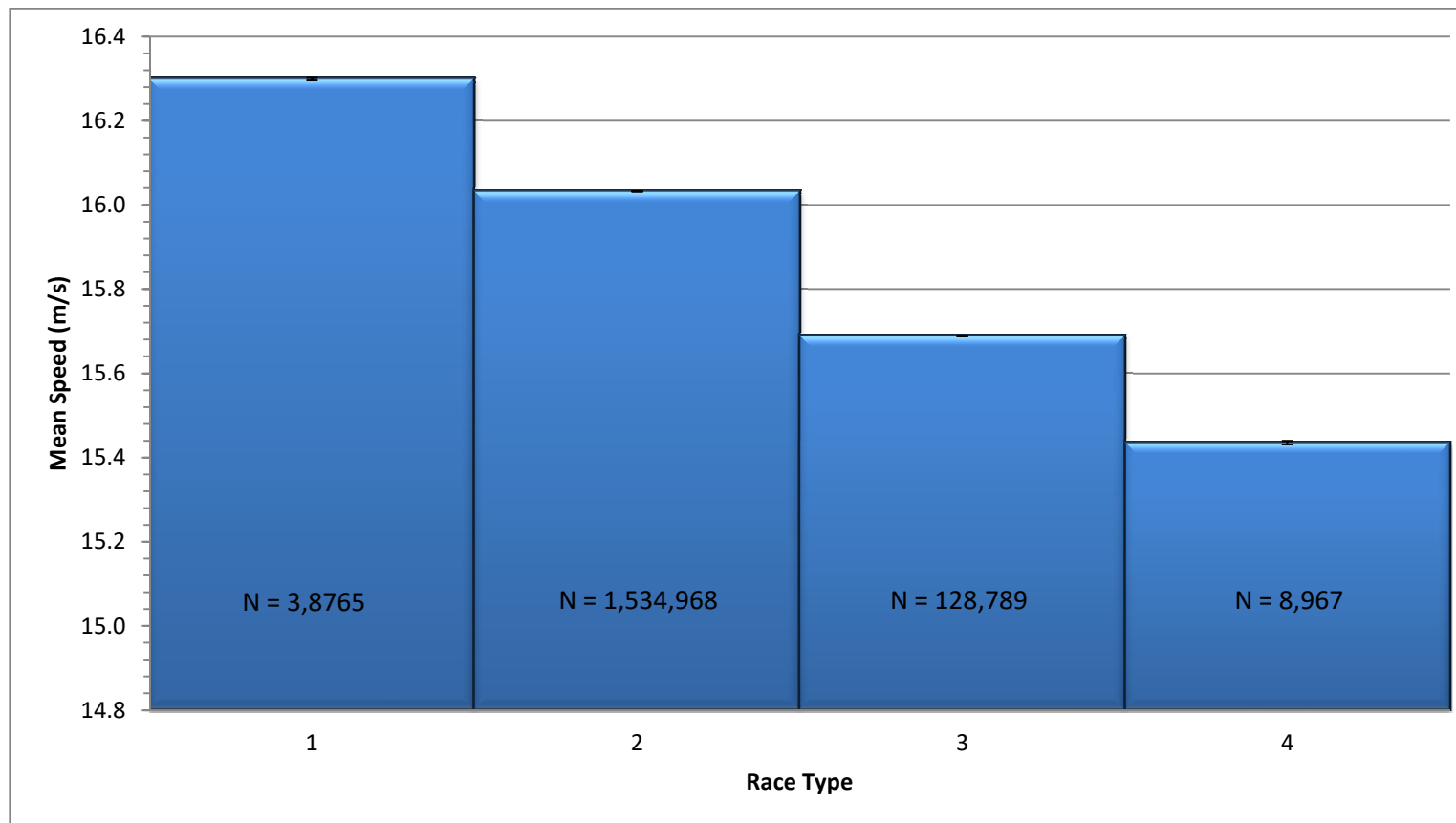


Figure 3.14. The mean racing speed (m/s) of British Greyhounds ($n = 1,711,489$ performance results) over all race distances (220 m to 1,080 m) during a five-year period from 2008 to 2012, according to race type. Where: Race Type 1 = race distances ≤ 305 m; Race Type 2 = race distances > 305 m and < 539 m; Race Type 3 = race distances ≥ 540 m and ≤ 700 m; Race Type 4 = race distances > 700 m. Error bars indicate the standard error of the mean.

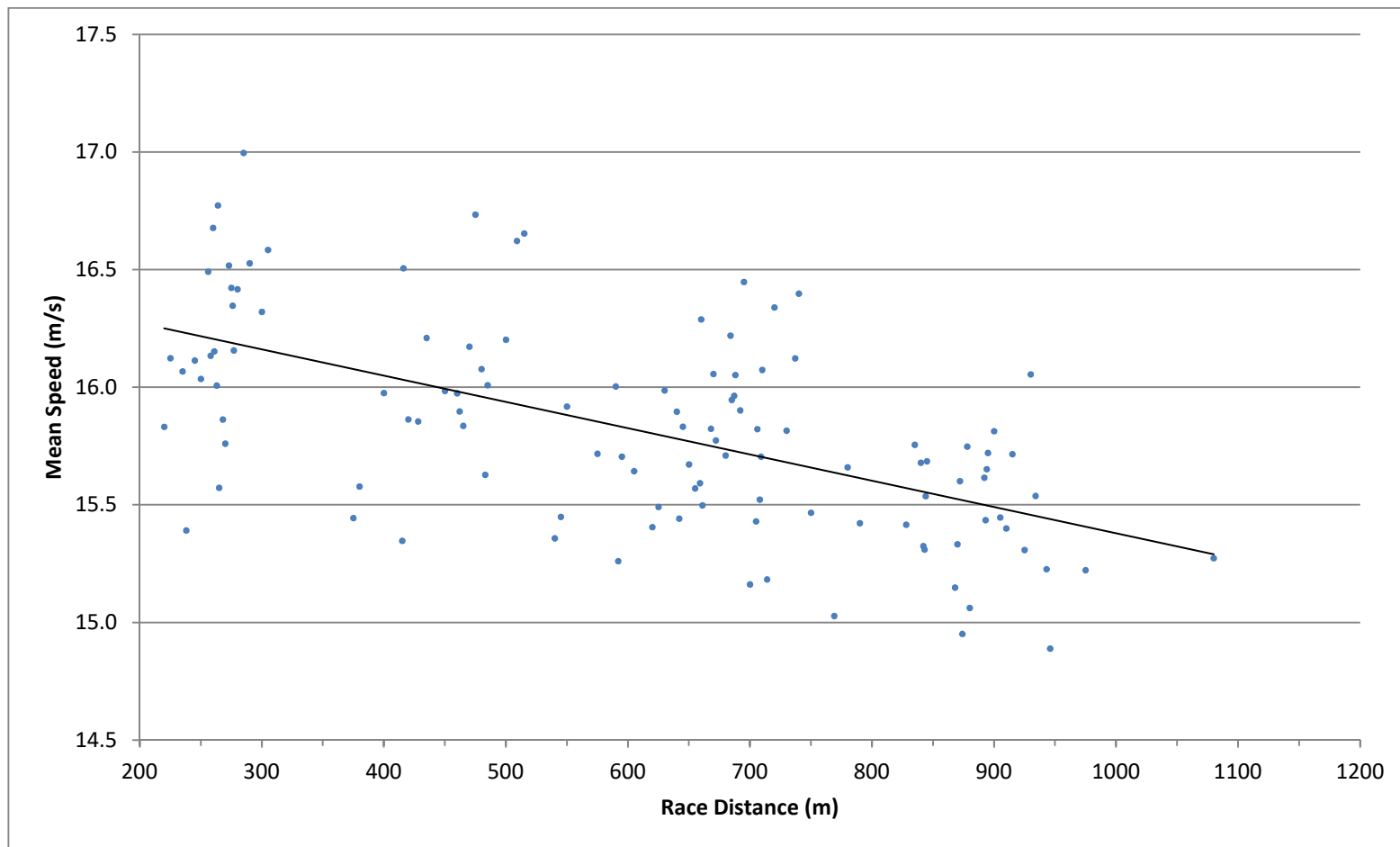


Figure 3.15. The mean racing speed (m/s) of British Greyhounds ($n = 1,711,489$ performance results) over a five-year period from 2008 to 2012, according to race distance (220 m to 1,080 m).

3.5. Discussion

The study aimed to investigate various environmental (non-genetic) influences on British racing Greyhound performance through analysis of the largest data set of Greyhound phenotypic information and race performance results to date, consisting of a total of 1,711,489 race performance records for 50,452 individual Greyhounds.

3.5.1. Sex of the Greyhound (Sex)

The data set consisted of 55.52% ($n = 950,184$) male and 44.48% ($n = 761,305$) female Greyhound performance results. This is similar to the male:female ratio reported by Taubert *et al.* (2007) for Greyhounds racing in Ireland, in which 54% of dogs were male and 46% female. In the present study, the sex of the Greyhound was found to be significantly associated with each of the performance traits Race Time ($p < 0.0001$), Speed ($p < 0.0001$) and Rank ($p < 0.0001$). Male dogs achieved lower mean race times, faster mean speeds and better mean rank finish positions compared to females. The results of the study are similar to those reported by Taubert *et al.* (2007) for racing Greyhounds in Ireland over a 480 m race distance, and Meissen (1997) for the racing performance of Whippets. Taubert *et al.* (2007) reported a statistically significant effect of sex ($p \leq 0.001$) on both Race Time and Rank, with least-squares mean values of 30.03 s for males and 30.15 s for females for Race Time, and of 3.35 for males and 3.56 for females for Rank. Ryan (1976), however, found no significant effect of sex on the racing performance of

Greyhounds in Ireland over a 480 m race distance. The influence of gender on the racing performance of British Greyhounds has not previously been reported.

Several studies have reported a statistically significant association between sex and race performance in Thoroughbred racehorses (Ekiz *et al.*, 2005a,b; Mota *et al.*, 1998, 2005; Svobodova *et al.*, 2005), with males achieving faster race times (Ekiz *et al.*, 2005a,b; Mota *et al.* 1998, 2005) and higher earnings (Ekiz *et al.*, 2005b; Svobodova *et al.*, 2005) compared to females. Oki *et al.* (1994), however, reported mixed results on dirt and turf tracks, with females faster than males on turf tracks, and males faster than females on dirt tracks for all distances except 1,200 m. Bakhtiari and Kashan (2009) found no significant effect of sex on Race Time and Rank in the Iranian Thoroughbred horse.

In the present study, the differences in speed and race performance between sexes is likely to reflect differing physiological characteristics of male and female dogs (Jelinek, 1988). In addition, there are reported variations in female Greyhound performance according to their stage of oestrus cycle. Payne (2013b) identified a decline in Greyhound racing performance between 41 and 56 days post-oestrus, with a gradual return to baseline performance levels by approximately 80 to 100 days post-oestrus. In the UK, female Greyhounds are not permitted to take part in a race or trial at a licensed stadium for a minimum period of 21 days from the start of oestrus (Greyhound Board of Great Britain, 2014). Payne (2013b), however, suggested that a minimum post-oestrus rest period of 70 days may be more appropriate. It is common practice in the UK to suppress

oestrus in female Greyhounds using hormonal medications, in order to prevent the decline in performance associated with oestrus and to avoid the requirement for time off from racing (Greyhound Board of Great Britain, 2009; Payne, 2013b). Licensed veterinary medicines available for the suppression of oestrus in bitches may be inappropriate for use in racing Greyhounds due to their anecdotal effects on Greyhound health, welfare and racing performance (Greyhound Board of Great Britain, 2014; Payne, 2013b). Consequently, oestrus is frequently suppressed in racing bitches using the human medication norethisterone, a synthetic form of the hormone progesterone, which is prescribed by a veterinary surgeon under the Veterinary Medicines Directorate (VMD) Cascade system. There have been no reported studies, however, investigating the effects of long-term season suppression on the health and welfare of the racing Greyhound.

The present study has identified considerable differences in Greyhound speed and racing performance between sexes. There are, however, similar numbers of male and female dogs competing in licensed races in the UK. During the study period of 2008 to 2012, male dogs accounted for 950,184 (55.52%) race performance results and female dogs accounted for 761,305 (44.48%) results. It appears, therefore, that the current demand for female Greyhounds within the British racing industry is relatively high.

In the UK, licensed Greyhound races are graded so that dogs of similar abilities, both male and female, are competing against each other. Each dog taking part in a race should have a reasonable chance of winning, regardless of sex, in order to

increase the difficulty of betting. Consequently, competitive mixed male and female Greyhound races are possible despite the overall performance variations between sexes.

A limitation of each of the models is that they assume each race start is independent. There is, however, clustering of the data, as the same dog can appear more than once in the performance results for each race start it completes during the study period.

3.5.2. Greyhound Birth Year (BirthYrInd)

The birth year of the dog was found to be significantly associated with each of the performance traits Race Time ($p < 0.0001$), Speed ($p < 0.0001$) and Rank ($p < 0.0001$), with Greyhounds born later performing better. Figure 3.3 demonstrates an increase in Greyhound mean speed with each successive birth year from 2001 to 2010. This indicates that there is likely to be genetic improvement in this trait within the Greyhound population over time, as a result of the selective breeding practiced. British Greyhounds usually start their racing career at 15 to 18 months of age. Consequently, the apparent decline in mean speed for Greyhounds born in 2011 may be explained by the fact that these dogs were not yet born or had not yet started racing at the time of data collection from 2008 to 2011, and would have been at the start of their racing career and therefore relatively inexperienced at the time of data collection in 2012. The influence of birth year on Greyhound racing performance has not previously been reported.

3.5.3. Greyhound Racing Age (RacingAge)

The racing age of British Greyhounds ranged from 15 months (1.25 years) to 98 months (8.16 years) of age at the time of the race, with a mean racing age of 36 months (three years). Of the total races, 16% involved Greyhounds aged two years or younger; 57% were by Greyhounds aged three years or younger; 86% involved Greyhounds aged four years or younger; and overall 97% of races were by Greyhounds aged five years or younger at the time of the race. Taubert *et al.* (2007), however, reported a considerably different age distribution of racing Greyhounds in Ireland, with over 50% of Greyhounds aged two years or younger, and in total over 90% of Greyhounds were aged three years or younger at the time of the race.

In the present study, racing age was found to be significantly associated with the traits Race Time ($p < 0.0001$) over 480 m, Speed ($p < 0.0001$) and Rank ($p < 0.0005$) over all distances. Faster mean speeds and lower race times were observed for male Greyhounds of all ages compared to females. Both male and female racing Greyhounds improved in speed from the start of their racing career to approximately 25 months of age, then decreased gradually in speed at similar rates over the next 45 months. Similarly, both male and female Greyhounds improved in race time from the start of their racing career to approximately 30 months of age, then increased gradually in race time (declined in performance) at similar rates over the next 40 months. The lower age of maximum performance for

the trait Speed may be explained by the inclusion of longer distance races (up to 1,080 m) in the data set. As Greyhounds gain more experience of racing, with increasing age, they may start to compete in races over longer distances in which lower mean speeds would be expected.

Experience of the Greyhound affects skill development and race performance. Similar to skill performance development in other animal species and in humans, the initial performance of a Greyhound is low at the start of their racing career, then increases over time with growth, adaptation and learning until reaching a peak. Performance then decreases with advancing racing age as a result of senescence or physiological deterioration. Helton (2009) examined changes in running performance of 14 elite racing Greyhounds in the USA, whilst controlling for changes in body weight, and found that a Greyhound's running skill development and performance exhibited marked improvement with increasing race experience. Greyhounds reached peak performance levels at an average of 2.4 years of age (Helton, 2009), with the time from initiation of racing career to achieving peak performance representing 9.1% of the average species lifespan of 8.2 years (Hayashidani *et al.*, 1988). Helton (2009) concluded that the length of time required for skill development relative to species lifespan is similar between canine and human sprint track athletes. In the present study, British Greyhounds reached peak performance at a slightly earlier mean age of 2.08 years.

Similar results have been reported by Ryan (1976) and Taubert *et al.* (2007) for racing Greyhounds in Ireland, and by Meissen (1997) for racing Whippets. Taubert

et al. (2007) reported a statistically significant effect of racing age on the traits Racing Time ($p \leq 0.001$) and Ranking ($p \leq 0.01$) for racing Greyhounds in Ireland over a 480 m distance, with Greyhounds of both sexes improving their race time until 30 months of age, remaining stable for 10 months, and then declining in speed from 40 months of age. In contrast to the present study, however, Taubert *et al.* (2007) reported that female Greyhounds demonstrated a steeper increase in race time (decline in speed) than males. This may be due to higher selection intensity in male Greyhounds compared to females, as a larger number of females are required for breeding than males (Taubert *et al.*, 2007). As a result, fast older male dogs would continue racing, whereas a large proportion of fast older females are likely to retire from racing and be used for breeding. The differences in decline in speed between male and female British and Irish Greyhounds may be explained by variations in the racing industries of the two countries. A large number of racing Greyhounds are bred in Ireland, to supply both the Irish and the British racing industries. In contrast, British-bred Greyhounds account for only 25% of Greyhounds racing in the UK (APGAW, 2007; Donoughue, 2007). Consequently, a considerably larger number of Greyhounds would be required for breeding purposes in Ireland, compared to the UK. As Greyhounds are traditionally bred based on phenotypic racing performance, the faster older females may be selected for breeding in Ireland and therefore retired from racing once they have reached peak performance. British Greyhound breeding, however, takes place on a considerably smaller scale. Consequently, the fast older British females may be less likely to retire from racing for breeding purposes after reaching peak

performance, and more likely to continue their racing careers in a similar manner to the older male dogs.

Several authors have reported a statistically significant association between age and racing performance in the Thoroughbred horse (Ekiz and Kocak, 2007; Mota *et al.*, 2005; Oki *et al.*, 1994); the trotter horse (Thiruvankadan *et al.*, 2009b); and dressage performance of sport horses (Stewart *et al.*, 2010). Mota *et al.* (2005) reported that excluding the 1,100 m distance, Thoroughbred horses of four years of age were significantly faster (0.13 s) than horses of other ages for all distances analysed (1,000 m, 1,200 m, 1,300 m, 1,400 m, 1,500 m and 1,600 m), with considerable superiority of 0.164 s and 0.22 s at greater distances of 1,500 m and 1,600 m, respectively. In contrast, Mota *et al.* (1998) reported a non-significant effect of age on racing performance of the Thoroughbred horse. This study, however, considered only the racing time of winning horses.

3.5.4. Number of Dogs in the Race (*N*dogs)

The number of dogs (4 - 6) in the race was found to be significantly associated with the performance traits Race Time ($p < 0.0001$) and Speed ($p < 0.0001$). The effect of *N*dogs on the performance trait Rank was not analysed, as all rank finish positions were adjusted prior to analysis (*AdjRank*) as detailed in Chapter 2.1.3.5, to account for races with differing numbers of participants.

Fastest mean speeds were observed when five dogs were competing in a race. This may be due to reduced crowding of the Greyhounds on the track with five-dog compared to six-dog races. Collisions or bumps between dogs are common during a race, particularly at the first bend where crowding tends to be greatest. Such incidents will often affect the dog's performance in the race, and may potentially result in injury. Consequently, having fewer numbers of dogs in the race will reduce crowding and the risk of collision or interference by other Greyhounds, which may result in greater race performance.

Slowest mean speeds, however, were observed when there were four dogs in a race. It may be expected that races consisting of only four Greyhounds would result in greater individual dog performance compared to five-dog or six-dog races, as there is less chance of crowding, collision or dog interference during the race. One possible explanation of this result is that Greyhounds are very competitive, with a strong instinct to chase and catch their prey. With only four dogs in a race there may be less perceived competition between dogs to chase and 'catch' the artificial lure. Consequently, this may result in slower racing speeds. The influence of number of dogs in the race on Greyhound racing performance has not previously been reported.

3.5.5. Starting Trap Number (Trap)

The starting trap number (1 - 6) was found to be significantly associated with the traits Race Time over 480 m ($p < 0.0001$), Speed ($p < 0.0001$) and Rank ($p < 0.0001$)

over all distances. At all Greyhound stadiums in the UK, starting trap one is nearest to the inner rail and trap six is nearest to the outer perimeter of the track. Racing Greyhounds will often display a preference for where they wish to run on the track; some preferring to run close to the inner rail and others preferring to run towards the middle or 'wide'. A wide-running Greyhound placed in starting trap one, for example, will often cross over to the other side of the track almost immediately after release from the trap, therefore risking collision with other dogs during high-speed acceleration. The allocation of starting trap number by the Racing Manager therefore takes into consideration the preference of the Greyhound for where they tend to run on the track, in order to minimise the risk of collision and injury to the dogs. An exception to this is a 'draw for traps' race, in which starting trap numbers are randomly allocated.

In the present study, Greyhounds achieved fastest mean speeds and best mean rank positions when starting from trap number one. Greyhounds starting from trap number five had slowest mean speeds and worst mean rank values. In any one race, the dogs running closer to the inner rail would effectively run a shorter race distance compared to those running towards the middle or outer perimeter of the track. This may account for the increased mean speed and better mean rank position of Greyhounds starting from trap one. Dogs starting from trap six, closest to the outer perimeter of the track, would effectively run a longer race distance compared to other dogs in the race if they continued to run close to the outer rail. Trap six, however, was associated with the second fastest mean speeds. A possible explanation of this result is that dogs running close to the inner or outer rail of the

track may encounter less interference or collisions with other dogs, compared to those running towards the centre of the track with other Greyhounds running either side. Another reason may be the location of the artificial lure, which runs around the inner rail or outer rail of the track, depending on stadium. Dogs starting the race from a position nearest to the artificial lure, therefore from trap one or trap six, depending on stadium, may demonstrate faster mean speeds due to their proximity to the 'prey'. A third possible explanation is that due to the non-random allocation of Greyhound to starting trap number by the Racing Manager, Greyhounds with a clear preference for running very close to the rails or very wide towards the outer perimeter of the track may be more likely to receive their preferred starting trap of one or six, respectively. The Greyhounds with a preference for running in the middle of the track may be considered more ambiguous, and therefore allocated any of starting traps two to five.

The influence of starting trap number on the racing performance of Greyhounds has not previously been reported.

3.5.6. Greyhound Stadium (StadiumID)

The stadium of the race was found to be significantly associated with Race Time ($p < 0.0001$) and Speed ($p < 0.0001$), in concordance with the findings of Ryan (1976) and Taubert *et al.* (2007) investigating race performance of Greyhounds in Ireland, and of Meissen (1997) investigating racing performance of whippets. Greyhounds in the UK race in an anticlockwise direction around an elliptical track. In the present study, a 1.33 m/s difference in speed between the fastest and slowest race tracks was observed. In the 12 tracks holding races over a 480 m race distance, there was a 0.97 s difference in race time between the fastest and slowest tracks. Similar results were obtained in the study by Taubert *et al.* (2007), which reported a 0.8 s difference in race time over a 480 m distance between the fastest and slowest race tracks in Ireland. In a study of racing Whippets, Meissan (1997) reported a difference in race time of 2.38 s between fastest and slowest tracks.

In the present study, a possible explanation of the observed difference in mean speeds between tracks is that British Greyhound stadiums vary considerably in terms of track design, circumference, radius of the bends, angles of inclination, track surface conditions and preparation methods; all of which may affect the racing speed of the dogs. The radius of a bend and degree of banking will affect the speed at which a Greyhound negotiates a bend, and the energy used by the dog (Cook, 1998; Gillette and Zebas, 1991). All Greyhound stadiums in the UK use sand-based surface material, however track surface depth, drainage systems,

preparation techniques and surface conditions vary considerably between tracks. Studies of racehorse stadiums have found that track surface conditions, such as substrate type, bulk density and moisture content, can influence performance by affecting racing speed (Field *et al.*, 1993; Zebarth and Sheard, 1985).

In the present study, the stadium of the race was found to have no significant effect on Rank ($p > 0.05$). This result is to be expected, as the rank finish position of a Greyhound is relative to the other dogs competing in that individual race, irrespective of overall speed or ability. All Greyhounds in a race are ranked from one to six, therefore similar numbers of first, second, third, fourth, fifth and sixth finish positions would be expected overall at a particular race track.

3.5.7. Effect of Season and Stadium (MYStad)

The influence of season, climatic conditions and variation between stadiums on racing performance was examined through analysis of the effect of month-year-stadium combination of the race, *MYStad* (1,509 levels). The variable *MYStad* was found to be significantly associated with the traits Race Time ($p < 0.0001$) over 480 m, Speed ($p < 0.0001$) and Rank ($p < 0.0001$) over all distances. Figure 3.13 displays the monthly mean racing speed across all race distances and the monthly mean temperature in the UK, indicating a close association between the two. Mean racing speeds were found to be slower in winter months (October to March) when ambient temperatures were lower, and faster in summer months (April to September) when temperatures were higher. Similar seasonal variation in mean

racing time over a 480 m distance was reported by Taubert *et al.* (2007) for racing Greyhounds in Ireland.

The results of the present study may be explained by the physiology of skeletal muscle, whereby muscles work more effectively and more efficiently when they are warmer (Syme, 2005). In order to improve racing performance particularly in colder months, and potentially reduce the risk of injury (Blythe *et al.*, 2007; Strickler *et al.*, 1990), it is therefore important to ensure adequate warm-up of the Greyhound prior to racing. In addition, during colder periods it may be beneficial for the stadium to provide heated kennels for the Greyhounds, in order to maintain warmth of their skeletal musculature prior to specific pre-race warm-up procedures by the trainer.

Variations in the consistency of the track surface, as a result of seasonal climatic conditions and changes in rainfall, may also affect the racing speed of the dog. Track conditions and racing speeds are thought to be affected by water content of the sand surface material, with the track running faster following light rain (or manual watering), but slower after heavier rainfall when the track becomes waterlogged (Cook, 1998; Iddon *et al.*, 2014). Further work could involve the collection of monthly rainfall data over the study period of 2008 to 2012, according to stadium location. This information could be used to update the existing database, to enable additional analysis of the effect of seasonal rainfall on Greyhound racing performance.

3.5.8. Race Distance (RaceDist) and Type of Race (RaceType)

In the present study, race distances varied considerably both within and between race tracks. The actual distance of the race (220 m - 1,080 m) was found to be significantly associated with Speed ($p < 0.0001$), with a decline in mean speed as the race distance increases. A difference in mean speed of 2.11 m/s between the fastest and slowest race distances was observed. Additionally, races were classified into four different Race Types according to race distance and number of bends encountered on the elliptical track. The type of race (1 - 4) was found to be significantly associated with Speed ($p < 0.0001$), with a decrease in mean speed with increasing race distance and number of bends to negotiate during the race. Racing Greyhounds are sprint animals, able to accelerate to speeds of up to 18 m/s in just a few seconds (Dobson *et al.*, 1988). They are able to maintain this maximum speed, however, for only the first 250 m of a race. Following this, racing speed declines gradually for the remainder of the race due to increasing fatigue.

Both race distance and race type were found to have no significant effect on Rank ($p > 0.05$). This result is to be expected, as the rank finish position (1 - 6) of a Greyhound is relative to the other dogs competing in that individual race, irrespective of race distance or type.

3.5.9. Conclusions

In conclusion, in the largest study of its kind, the present study has identified a number of environmental (non-genetic) factors that are significantly associated with racing performance in British Greyhounds. The environmental factors of sex of the dog, Greyhound birth year, number of dogs in the race, starting trap number, stadium, month-year-stadium combination of the race and racing age of the dog were each found to be significantly associated with the performance traits Race Time over a 480 m distance and Speed over all distances. In addition, the race type and distance of the race were significantly associated with Speed over all distances. The factors of sex of the dog, Greyhound birth year, starting trap number, month-year-stadium combination of the race and racing age of the dog were significantly associated with Rank, whereas factors of race type, race distance and stadium had no significant effect on Rank.

The results of the study demonstrate an overall phenotypic improvement in the race time of British Greyhounds over a 480 m race distance during the five-year study period from 2008 to 2012 (Figure 3.12), from a mean race time of 29.98 s to 29.74 s (improvement of 0.24 s). Similarly, there was an overall phenotypic improvement in racing speed of British Greyhounds during the five-year period (Figure 3.13), from a mean speed of 15.96 m/s to 16.05 m/s (improvement of 0.09 m/s). This is equivalent to an improvement of 0.048 seconds per year in Race Time and 0.018 metres per second per year in Speed. Taubert *et al.* (2007) reported a

higher phenotypic improvement of 0.125 seconds per year for Race Time in Irish Greyhounds over a distance of 480 m, during a four-year period from 2000 to 2003. It is not possible to attain a phenotypic improvement in Rank within the population, as a Rank of one (first) is always the highest.

In Chapter Four, the results of this study will be used in combination with pedigree data to perform a genetic evaluation of race performance in British Greyhounds. Relevant environmental factors identified as significantly associated with racing performance will be accounted for in the calculation of variance components, estimated heritabilities and repeatabilities for the three performance traits, and in the calculation of EBVs for individual dogs.

Chapter Four.

Genetic Analysis of Race Performance in British Greyhounds

4. Genetic Analysis of Race Performance in British Greyhounds

4.1. Introduction

4.1.1. Production of Greyhounds for the Racing Industry

Greyhound racing is popular in the UK, both as a spectator sport and as an off-course betting medium. Over the past decade, however, the welfare of British racing Greyhounds has received increased public and political interest. In particular, concern has been expressed regarding the number of Greyhounds that seem to 'go missing' before the start of their racing careers. The APGAW (2007) inquiry into the welfare of racing Greyhounds in England, as well as an independent review of the British Greyhound industry (Donoughue, 2007), highlighted that the industry produces a surplus of unwanted young dogs that do not make the grade to race. An estimated 6,000 to 12,000 young Greyhounds bred to supply the UK racing industry each year did not ever enter competitive racing, and were unaccounted for (APGAW, 2007). It is likely that the majority of these Greyhounds initially entered training programmes, but were later discarded due to inadequate performance, behavioural problems or disinterest in racing (APGAW, 2007). With no accurate data available to establish what happened to them, it is likely that a considerable number may have been euthanased (APGAW, 2007).

The reports of the APGAW (2007) inquiry and independent review (Donoughue, 2007) of the British Greyhound industry both recommend that urgent action is required to address the significant welfare issue of over-breeding and over-supply

of young Greyhounds for the racing industry. The majority (75%) of Greyhounds that race in the UK were originally bred in Ireland and later imported into the UK (APGAW, 2007). Current European Union trade regulations prevent import restrictions on the numbers of Irish-bred Greyhounds entering the UK. The British Greyhound industry was strongly advised, however, to consider the standards of breeding and the selection of traits for which Greyhounds are bred (APGAW, 2007; Donoghue, 2007).

Over recent years, the British Greyhound racing industry and UK Government have taken substantial measures to improve the general welfare and traceability of racing Greyhounds in the UK. One of the most significant issues yet to be addressed, however, is the number of surplus young Greyhounds produced by the industry each year.

4.1.2. Selection and Animal Breeding

For centuries, Greyhounds have been subjected to intense selective breeding in an attempt to produce faster and better-performing dogs (Denny, 2008). The selection of Greyhounds for breeding, however, is currently based on their own or their relative's phenotypic (observed) racing performance; a slow and inefficient method of animal improvement (Taubert *et al.*, 2007). The objective of animal breeding is to accurately identify animals with 'superior' characteristics, and to select these individuals for breeding. Over the past 50 to 60 years, scientific methods of selection based on performance measurements and genetic

evaluations have become widely used in dairy and beef cattle, pig, poultry and sheep breeding; resulting in increased output, decreased costs, and improvements in the quality of animal products (Simm, 1998).

In animal breeding, the majority of traits of economic importance are quantitative traits controlled by many different genes at different loci, and are also affected by non-genetic (environmental) influences. Modern animal breeding programmes aim to separate the effects of genotype and the environment, to enable the selection of animals with high genetic merit rather than those that perform well as a result of favourable environmental conditions. Methods of quantitative genetic analysis can be used to quantify and adjust records of performance for known environmental influences, and calculate EBVs for individual animals (Simm, 1998). The EBV estimates the proportion of superiority or inferiority in an individual's performance that is due to additive genetic effects, therefore enabling individual animals to be ranked according to their additive genetic merit. Targeted genetic selection based on EBVs enables breeders to produce animals of superior and predictable genetic merit, therefore minimising the over-breeding and wastage of young animals that do not perform as expected or required. The use of EBVs in the selection of individuals for breeding has been extremely successful in improving health, production, performance and trainability in many groups of animals, including sports horses, dairy cattle, beef cattle, guide dogs, pigs, poultry, sheep and fish (Bourdon, 2014; Famula, 2012; Simm, 1998).

In contrast to other animal groups, there are only three reported genetic studies investigating racing performance of the Greyhound (Desgorces *et al.*, 2012; Ryan, 1975; Taubert *et al.*, 2007). Two of these studies analysed the performance of Greyhounds racing in Ireland. Importantly, there have been no previous genetic studies investigating the racing performance of British Greyhounds, and EBVs have not previously been calculated.

4.1.3. Calculation of Estimated Breeding Value (EBV)

The EBV of an animal is obtained by measurement of phenotypic performance of the individual and/or its relatives for a particular trait, and that of the population group in which it performs (Nicholas, 2003; Simm, 1998). An estimation of the h^2 of the trait being measured is required. A detailed performance recording system, accurate identification of individual animals and accurate pedigree records are essential.

In the simplest case, the EBV of an individual can be predicted using a single record of their own performance. Following adjustment of performance records to take account of relevant environmental influences, the EBV is calculated as the deviation in performance of the individual (P_x) from the contemporary group (P_{pop}), multiplied by the h^2 of the trait concerned:

$$EBV = h^2 \times (P_x - P_{pop}) \quad (\text{Simm, 1998})$$

Using repeated records of performance from the same individual can increase the accuracy of breeding value estimation for that individual, and can increase the response to selection, depending on the repeatability of the trait concerned. Repeatability is defined as the proportion of the total phenotypic variation (σ^2_p) in a trait which is explained by the combined effects of additive (σ^2_{GA}) and non-additive (σ^2_{GNA}) genetic variation and permanent environmental variation (σ^2_{Ep}). This can be expressed as:

$$\text{Repeatability} = \frac{\sigma^2_{GA} + \sigma^2_{GNA} + \sigma^2_{Ep}}{\sigma^2_p}$$

(Simm, 1998)

Repeatability measures the similarity between repeated performance records of the same trait, assuming that the performance measured at different times is controlled by the same genes. Similar to heritabilities, repeatabilities range from zero to one or from 0% to 100%. The higher the repeatability of a trait, the lower the value of using repeated records of performance from an individual in selection for that trait (Simm, 1998).

The EBV of an individual can therefore be predicted using repeated records of their own performance. Following adjustment of performance records to take account of relevant environmental influences, the EBV is calculated as the mean deviation in performance of the individual (\bar{P}_x) from the mean of the contemporary group

(\bar{P}_{pop}), multiplied by a regression coefficient (b) which depends on the number of repeated records and on the h^2 and repeatability of the trait concerned:

$$EBV = b \times (\bar{P}_x - \bar{P}_{pop}) \quad (\text{Simm, 1998})$$

In addition, the EBV of an individual can be predicted using performance records of their relatives. In the simplest case, using records of performance from the parents of the individual, the EBV of their offspring is calculated as:

$$EBV \text{ of Offspring} = \frac{1}{2}EBV \text{ of Sire} + \frac{1}{2}EBV \text{ of Dam} \quad (\text{Simm, 1998})$$

If the EBV of one parent is unknown, the EBV of the offspring is calculated as half the EBV of the known parent. Other relatives can also be used in the prediction of breeding values, by taking into account the proportion of genes in common between an individual and their relatives (Table 4.1).

Class of Relative	Proportion of Genes in Common with Individual X
Individual X	1
Parent	0.5
Grandparent	0.25
Great-Grandparent	0.125
Identical Twin	1
Full Sibling	0.5
Half Sibling	0.25
Progeny	0.5

Table 4.1. The proportion of genes in common between Individual X and various classes of relatives.

In the simplest case, using a single record of performance from any relative, the EBV of the related individual is calculated as:

$$\text{EBV} = \text{Proportion of genes in common with the relative} \times \text{EBV of relative} \quad (\text{Simm, 1998})$$

The prediction of breeding values using a combination of performance records from different classes of relatives becomes more complex. One method involves the production of an index, with weighted contributions from different classes of relatives according to the proportion of genes in common and the number of records available for each relative class.

4.1.4. The Selection Index

Selection index theory was first developed in the 1930s and 1940s as a tool for genetic prediction using performance data from genetically similar contemporary groups (Bourdon, 2014). Following adjustment of performance records for relevant environmental influences, selection indexes are used to combine records of performance for a single trait, or several different traits, measured on the individual itself and one or more classes of relative (Simm, 1998). A selection index is a linear combination of phenotypic performance data and suitable weighting factors, and it takes the following form:

$$I = b_1P_1 + b_2P_2 + \dots + b_nP_n \quad (\text{Bourdon, 2014; Simm, 1998})$$

Where:

I = the index score for an individual

b_i = a weighting factor or index coefficient, which is dependent on the h^2 of the trait under selection, the class of relative concerned and the number of performance measurements available for each class of relative

P_i = the measurement of phenotypic performance on the individual itself, or the average measurement from different classes of relatives, after adjustment for known environmental influences

n = the total number of measurements of phenotypic performance

4.1.5. Best Linear Unbiased Prediction (BLUP)

Best Linear Unbiased Prediction (BLUP) was first proposed in 1949 by Professor C. R. Henderson at Iowa State and Cornell Universities in the USA. BLUP is a statistical technique which efficiently disentangles genetic from non-genetic (environmental) effects, in order to estimate environmental influences and predict EBVs simultaneously. BLUP enables performance records of the individual animal and/or those of its relatives to be combined, taking into account the relationships between them, in order to produce more accurate predictions of EBVs in one step (Nicholas, 2003; Simm, 1998). Sire-model BLUP evaluations predict EBVs for sires using their progeny records, and were first used in the USA in the early 1970s to

produce EBVs for dairy cattle. These evaluations were later introduced to the UK dairy industry in 1979 (Simm, 1998). Advances in computing enabled the introduction of more sophisticated individual animal-model BLUP evaluations in 1992. These evaluations predict EBVs for each individual included in the analysis by taking into account all familial relationships between them, through the inclusion of the numerator relationship matrix (**A**) calculated from the pedigree data (Henderson, 1976). Consequently, predictions are available for both males and females, parents and non-parents, ancestors, offspring and animals not yet conceived, and animals with or without records of performance (Bourdon, 2014). BLUP has now become the most widely accepted method for large-scale genetic evaluation of animal populations (Bourdon, 2014; Simm, 1998). EBVs calculated by BLUP for individual traits are often weighted and combined to produce a selection index, in order to assist breeders in selection across several traits simultaneously.

4.1.5.1. *The Linear Mixed Model*

A mixed model is a statistical model containing both fixed and random effects. In matrix notation, a general mixed model can be represented as:

$$\mathbf{y} = \mathbf{X}\mathbf{b} + \mathbf{Z}\mathbf{a} + \mathbf{e}$$

Where:

\mathbf{y} = the $n \times 1$ column vector containing the observations of n individuals

\mathbf{b} = the $p \times 1$ vector of fixed effects, where p is the number of levels of fixed effects

\mathbf{a} = the $q \times 1$ vector of random effects, where q is the number of levels of random effects

\mathbf{e} = the $n \times 1$ column vector of residual deviations

\mathbf{X} = the $n \times p$ incidence matrix, relating the observations to \mathbf{b}

\mathbf{Z} = the $n \times q$ incidence matrix, relating the observations to \mathbf{a}

In animal breeding, the first element of vector \mathbf{b} is typically the population mean, and other factors may include, for example, sex of the animal, year of birth or location. The elements of the vector \mathbf{a} of random effects are usually random genetic effects such as additive genetic values, and \mathbf{e} is assumed to be distributed independently of the random genetic effects. Each element of the incidence matrices \mathbf{X} and \mathbf{Z} are usually equal to zero or one, depending on whether the relevant fixed or random effect contributes to the individual's phenotype (Lynch and Walsh, 1998).

Considering the means and variances (*var*) of the component vectors of the mixed model, the expectation (*E*) to the variables is: $E(\mathbf{a}) = E(\mathbf{e}) = 0$ by definition, therefore $E(\mathbf{y}) = \mathbf{Xb}$. The ($q \times q$) covariance (*cov*) matrix for the vector \mathbf{a} of random genetic effects can be denoted by \mathbf{G} , so that $var(\mathbf{a}) = \mathbf{A}\sigma_a^2 = \mathbf{G}$, where \mathbf{A} is the numerator relationship matrix. The ($n \times n$) covariance matrix for the vector of \mathbf{e} residual errors can be denoted by \mathbf{R} . It is generally assumed that residual errors have constant variance and are uncorrelated, so that $var(\mathbf{e}) = \mathbf{I}\sigma_e^2 = \mathbf{R}$, where \mathbf{I} is an identity matrix. It is assumed that \mathbf{a} and \mathbf{e} are uncorrelated so that $cov(\mathbf{a}, \mathbf{e}) = cov(\mathbf{e}, \mathbf{a}) = 0$. Excluding the difference between individuals due to fixed effects, the covariance matrix (\mathbf{V}) for the vector of observations \mathbf{y} can be represented as:

$$\mathbf{V} = var(\mathbf{Za} + \mathbf{e}) = \mathbf{ZGZ}^T + \mathbf{R}$$

(Lynch and Walsh, 1998)

Where \mathbf{Z}^T is the transpose of the matrix \mathbf{Z} , obtained by interchanging the rows and columns of the original matrix. For example:

$$\begin{pmatrix} 1 & 4 & 7 \\ 2 & 5 & 8 \\ 3 & 6 & 9 \end{pmatrix}^T = \begin{pmatrix} 1 & 2 & 3 \\ 4 & 5 & 6 \\ 7 & 8 & 9 \end{pmatrix}$$

4.1.5.2. *Estimation of Fixed Effects and Prediction of Random Effects*

Mixed model analysis involves estimation (or prediction) of the vectors of fixed and random effects, **b** and **a**, as well as estimation of the covariance matrices **G** and **R**.

In quantitative genetic analysis of linear mixed models, BLUP is the most commonly used procedure for the prediction of random effects and Best Linear Unbiased Estimator (BLUE) is used for the estimation of fixed effects. These procedures are 'best' in that they minimise the sampling variance, 'linear' as they are linear functions of the phenotypic observations **y**, and 'unbiased' in that $E[\text{BLUP}(\mathbf{a})] = \mathbf{a}$ and $E[\text{BLUE}(\mathbf{b})] = \mathbf{b}$ (Lynch and Walsh, 1998).

For the general mixed model described in Chapter 4.1.5.1, the BLUE of **b** can be calculated using the following equation:

$$\text{BLUE}(\mathbf{b}) = \hat{\mathbf{b}} = (\mathbf{X}^T \mathbf{V}^{-1} \mathbf{X})^{-1} \mathbf{X}^T \mathbf{V}^{-1} \mathbf{y}$$

The BLUP of **a** is calculated by:

$$\text{BLUP}(\mathbf{a}) = \hat{\mathbf{a}} = \mathbf{GZ}^T \mathbf{V}^{-1} (\mathbf{y} - \mathbf{X}\hat{\mathbf{b}}) \quad (\text{Henderson, 1963})$$

Where **V** is defined in Chapter 4.1.5.1. The solution of equations for BLUE(**b**) and BLUP(**a**) requires that the variance components, and therefore the covariance

matrices \mathbf{R} and \mathbf{G} , are known. Variance components must therefore be estimated prior to performing BLUP or BLUE analysis. The solution of the equations also requires the inverse of the covariance matrix \mathbf{V} . The calculation of \mathbf{V}^{-1} , however, is computationally demanding when \mathbf{y} contains large numbers of observations (Lynch and Walsh, 1998). Henderson (1950, 1963, 1973, 1984) therefore described a simpler method for calculation of $\hat{\mathbf{a}}$ and $\hat{\mathbf{b}}$ simultaneously, without the requirement of \mathbf{V}^{-1} , in the form of mixed-model equations (MME):

$$\begin{pmatrix} \mathbf{X}^T \mathbf{R}^{-1} \mathbf{X} & \mathbf{X}^T \mathbf{R}^{-1} \mathbf{Z} \\ \mathbf{Z}^T \mathbf{R}^{-1} \mathbf{X} & \mathbf{Z}^T \mathbf{R}^{-1} \mathbf{Z} + \mathbf{G}^{-1} \end{pmatrix} \begin{pmatrix} \hat{\mathbf{b}} \\ \hat{\mathbf{a}} \end{pmatrix} = \begin{pmatrix} \mathbf{X}^T \mathbf{R}^{-1} \mathbf{y} \\ \mathbf{Z}^T \mathbf{R}^{-1} \mathbf{y} \end{pmatrix}$$

Henderson (1950) obtained the MME by assuming that the co-variance matrices \mathbf{G} and \mathbf{R} are known, and that the densities of the vectors \mathbf{a} and \mathbf{e} are both multivariate normal and uncorrelated. The solution to the MME therefore gives the maximum likelihood estimates of the fixed and random effects. In later work, however, Henderson (1963) proved that the MME are not dependent on normality, and that $\hat{\mathbf{a}}$ and $\hat{\mathbf{b}}$ are BLUP(\mathbf{a}) and BLUE(\mathbf{b}), respectively, under general conditions when variance components are known (Lynch and Walsh, 1998).

4.1.5.3. *The Animal Model*

The 'animal model' is based on the general mixed model described in Chapter 4.1.5.1, and is used for calculation of EBVs for each measured individual. Under the simplest animal model and assuming only one fixed factor (the population mean, μ), the observation for individual i can be represented by the following equation:

$$y_i = \mu + a_i + e_i \quad (\text{Lynch and Walsh, 1998})$$

Where a is the additive genetic value of individual i , and e is the residual error.

The \mathbf{G} matrix describes the covariances between random genetic effects (the EBVs). The genetic covariance between two relatives i and j can be calculated as twice the coefficient of coancestry multiplied by the additive genetic variance (σ_a^2) in the base population. This can be expressed as:

$$2\Theta_{ij}\sigma_a^2 \quad (\text{Lynch and Walsh, 1998})$$

Under the animal model, therefore, $\mathbf{G} = \mathbf{A}\sigma_a^2$, where \mathbf{A} is the additive genetic (or numerator) relationship matrix and $\mathbf{A} = 2\Theta_{ij}$. It is generally assumed that $\mathbf{R} = \mathbf{I}\sigma_e^2$, where \mathbf{I} is an identity matrix. As $\mathbf{G}^{-1} = \mathbf{A}^{-1}\sigma_a^{-2}$ and $\mathbf{R}^{-1} = \mathbf{I}\sigma_e^{-2}$, the MME for the animal model can be expressed as:

$$\begin{pmatrix} \mathbf{X}^T\mathbf{X} & \mathbf{X}^T\mathbf{Z} \\ \mathbf{Z}^T\mathbf{X} & \mathbf{Z}^T\mathbf{Z} + \lambda\mathbf{A}^{-1} \end{pmatrix} \begin{pmatrix} \hat{\mathbf{b}} \\ \hat{\mathbf{a}} \end{pmatrix} = \begin{pmatrix} \mathbf{X}^T\mathbf{y} \\ \mathbf{Z}^T\mathbf{y} \end{pmatrix}$$

Where $\lambda = \sigma_e^2 / \sigma_a^2 = (1 - h^2) / h^2$, assuming additive gene action (Lynch and Walsh, 1998).

4.1.6. Estimation of Variance Components

Restricted maximum likelihood (REML) is the method of choice for estimation of variance components, and therefore h^2 , in large populations with complex but known pedigrees. REML is a modification of maximum likelihood estimation which adjusts the observations for estimates of the fixed effects, to produce unbiased estimates of variance and covariance parameters. This type of analysis does not require balanced designs, can accommodate any structure of genetic relationships in the data, and can take account of non-random selection of parents from the population (Falconer and Mackay, 1996). REML is available using general statistical software packages such as SAS (SAS Institute Inc., 2002-2010), as well as more specialist software packages such as DMU (Madsen and Jensen, 2013).

4.2. Aims

The aims of the study were to investigate genetic and environmental variation in race performance data of British Greyhounds; to calculate variance components, estimated heritabilities and repeatabilities for the industry-important performance traits Race Time, Speed and Rank; and to calculate EBVs of British Greyhounds for each of these traits, enabling individuals to be ranked according to additive genetic merit. Finally, the study aimed to identify the top 20 Greyhound sires and dams with respect to numbers of offspring produced within the data set, in order to compare their popularity as breeding animals with their additive genetic merit (EBV) for the three performance traits.

4.3. Materials and Methods

4.3.1. Data Collection and Cleaning for Quality Control

Greyhound phenotypic and historical race performance data were obtained for every Greyhound competing in each race that took place at GBGB-licensed British Greyhound stadiums during a five-year period from 1st January 2008 to 31st December 2012, using the publically available online resource Greyhound-Data (2014). In total, 1,882,658 race performance records for 52,826 individual Greyhounds were obtained and collated into a Microsoft Access database (Microsoft Corporation, 2007a), as detailed in Chapter 2.1. The maternal and paternal pedigree of each of the 52,826 individual Greyhounds with performance results were traced back six generations, producing a pedigree file containing a total of 73,346 Greyhounds.

Data cleaning, quality control steps and preparation of the pedigree file were performed as detailed in Chapter 2.1. The final data set (Appendix 1) for statistical and genetic analysis contained a total of 1,711,489 individual race performance records for 50,452 individual Greyhounds, with a total of 73,344 Greyhounds in the pedigree file.

4.3.2. Influence of Environmental (Non-Genetic) Factors on the Race Performance of British Greyhounds

Greyhounds are traditionally selected for breeding based on phenotypic performance in terms of racing speed, race time over a specific distance and/or rank finish position in a race (Taubert *et al.*, 2007). Consequently, these three measures of racing performance were selected for genetic analysis in the present study.

The influence of a number of environmental (non-genetic) factors on Greyhound race performance was investigated in Chapter Three, in order to determine the effects to consider in the final genetic analyses. Three separate general linear model analyses were performed for each of the three performance traits: Race Time over a 480 m race distance ($n = 481,882$ race performance results), Speed and Rank over all distances ($n = 1,711,489$ race performance results), as detailed in Chapter 3.3. The results of these analyses are summarised in Table 3.5.

4.3.3. Animal Model Genetic Evaluations

Univariate linear mixed animal model genetic evaluations were performed for each of the three performance traits: Race Time over a 480 m distance ($n = 481,882$ race performance results), Speed and Rank over all distances ($n = 1,711,489$ race performance results). Environmental (non-genetic) factors were considered for inclusion in each of the three animal models if they were found to

be significantly associated with the race performance trait under analysis, as detailed in Table 3.5.

4.3.3.1. Race Time

The following linear mixed animal model was used for estimation of genetic parameters and calculation of EBVs for the performance trait Race Time (over a 480 m distance):

$$y_{ijklmn} = \mu + \text{Sex}_i + \text{Ndogs}_j + \text{Trap}_k + b(\text{RaceAge}_l) + b(\text{RaceAge}_l^2) + b(\text{RaceAge}_l^3) \\ + \text{MYStad}_m + \text{pe}_l + a_l + e_{ijklmn}$$

(Model One)

Where y_{ijklmn} is the observation n of dog l (finish time in a given race); μ is the population mean; sex_i is the fixed effect of the i th sex ($i = 1, 2$); ndogs_j is the fixed effect of the j th number of dogs in the race ($j = 4, \dots, 6$); trap_k is the fixed effect of the k th starting trap number ($k = 1, \dots, 6$); $b(\text{raceage}_l)$, $b(\text{raceage}_l^2)$ and $b(\text{raceage}_l^3)$ are the regression coefficients of trait on racing age of animal l ; mystad_m is the random effect of the m th month-year-stadium of the race (1,509 levels); pe_l is the random permanent environmental effect of animal l ; a_l is the random additive genetic effect of animal l , $\sim \text{ND}(0, \mathbf{A}\sigma_a^2)$, assumed to be normally distributed (ND) where \mathbf{A} is the numerator relationship matrix and σ_a^2 is the additive genetic variance; and e_{ijklmn} is the random residual effect, $\sim \text{ND}(0, \mathbf{I}\sigma_e^2)$, where \mathbf{I} is an identity matrix and σ_e^2 is the residual variance.

4.3.3.2. Speed

The following linear mixed animal model was used for estimation of genetic parameters and calculation of EBVs for the performance trait Speed (over all race distances):

$$y_{ijklmno} = \mu + \text{sex}_i + \text{racetype}_j + \text{ndogs}_k + \text{trap}_l + b(\text{raceage}_m) + b(\text{raceage}_m^2) + b(\text{raceage}_m^3) + \text{mystad}_n + \text{pe}_m + a_m + e_{ijklmno}$$

(Model Two)

Where $y_{ijklmno}$ is the observation o of dog m (mean speed in a given race); μ is the population mean; sex_i is the fixed effect of the i th sex ($i = 1, 2$); racetype_j is the fixed effect of the j th race type ($j = 1$ [race distances ≤ 305 m], 2 [race distances > 305 m and < 539 m], 3 [race distances ≥ 540 m and ≤ 700 m], 4 [race distances > 700 m]); ndogs_k is the fixed effect of the k th number of dogs in the race ($k = 4, \dots, 6$); trap_l is the fixed effect of the l th starting trap number ($l = 1, \dots, 6$); $b(\text{raceage}_m)$, $b(\text{raceage}_m^2)$ and $b(\text{raceage}_m^3)$ are the regression coefficients of trait on racing age of animal $_m$; mystad_n is the random effect of the n th month-year-stadium of the race (1,509 levels); pe_m is the random permanent environmental effect of animal m ; a_m is the random additive genetic effect of animal m , $\sim \text{ND}(0, \mathbf{A}\sigma_a^2)$, with \mathbf{A} and σ_a^2 as previously defined; and $e_{ijklmno}$ is the random residual effect, $\sim \text{ND}(0, \mathbf{I}\sigma_e^2)$, with \mathbf{I} and σ_e^2 as previously defined.

4.3.3.3. Rank

The following linear mixed animal model was used for estimation of genetic parameters and calculation of EBVs for the performance trait Rank (over all race distances):

$$y_{ijklm} = \mu + \text{sex}_i + \text{trap}_j + b(\text{raceage}_k) + \text{mystad}_l + \text{pe}_k + a_k + e_{ijklm}$$

(Model Three)

Where y_{ijklm} is the observation m of dog k (rank in a given race); μ is the population mean; sex_i is the fixed effect of the i th sex ($i = 1, 2$); trap_j is the fixed effect of the j th starting trap number ($j = 1, \dots, 6$); $b(\text{raceage}_k)$ is the regression coefficient of trait on racing age of animal k ; mystad_l is the random effect of the l th month-year-stadium of the race (1,509 levels); pe_k is the random permanent environmental effect of animal k ; a_k is the random additive genetic effect of animal k , $\sim \text{ND}(0, \mathbf{A}\sigma_a^2)$, with \mathbf{A} and σ_a^2 as previously defined; and e_{ijklm} is the random residual effect, $\sim \text{ND}(0, \mathbf{I}\sigma_e^2)$, with \mathbf{I} and σ_e^2 as previously defined.

In the animal models one, two and three, the environmental factors of both season and stadium are accounted for by including the random effect of month-year-stadium combination of the race, *MYStad*. The environmental effect of birth year of the individual, *BirthYrInd*, was excluded from each of the three final models as it was found to pick up some of the genetic trend.

The environmental factor of race type [1 (race distances ≤ 305 m), 2 (race distances > 305 m and < 539 m), 3 (race distances ≥ 540 m and ≤ 700 m) or 4 (race distances > 700 m)] groups similar race distances together taking into account the number of bends to negotiate during the race. Overall, mean racing speed was found to decrease with increasing race distance. Within each race type category (1 - 4), however, there was no association between race distance and mean racing speed (Figure 3.15). Consequently, the environmental factor of race type was included in model two rather than the exact race distance.

The regression coefficients of trait on racing age of the Greyhound were included in models one and two, as previous analysis of the data in Chapter Three identified that a cubic regression provided a better fit for the data of mean race time over a 480 m distance (Figure 3.6) and for mean racing speed (Figure 3.5).

4.3.4. Estimation of Genetic Parameters and Breeding Values

The variance components and breeding values were estimated using the freely available software package DMU, version 6, release 5.2 (Madsen and Jensen, 2013). DMU is a package for analysis of mixed models and a powerful tool for estimation of variance components, estimation of fixed effects using BLUE and prediction of random effects by BLUP. The pedigree and Greyhound phenotype and performance data files were exported from SAS (SAS Institute Inc., 2002-2010) as two separate text files and then input to DMU (Madsen and Jensen, 2013) for

genetic analysis. The Greyhound pedigree file was used to define an additive genetic relationship.

4.3.4.1. *Estimation of Genetic Parameters*

For each of the animal models (1 to 3) defined in Chapter 4.3.3, variance components were estimated by univariate analysis using the Average Information Algorithm for Restricted Maximum Likelihood (AI-REML) (Jensen *et al.*, 1997), implemented by the DMU1 and DMUAI modules of the software package DMU (Madsen and Jensen, 2008). The AI-REML algorithm uses average information as second differentials of the likelihood function, obtaining average information through averaging the information matrices based on observed and expected data, and producing asymptotic standard errors of estimated (co)variance components (Madsen and Jensen, 2008) (Appendix 1).

Estimated h^2 is determined by the ratio of additive genetic variance (σ_a^2) to phenotypic variance (σ_p^2):

$$h^2 = (\sigma_a^2) / (\sigma_p^2) \quad (\text{Simm, 1998})$$

Phenotypic variance (σ_p^2) can be further divided into additive genetic variance (σ_a^2), permanent environmental variance (σ_{pe}^2) and residual variance (σ_e^2). For each of the three performance traits, estimated heritabilities were therefore calculated as:

$$h^2 = (\sigma_a^2) / (\sigma_a^2 + \sigma_{pe}^2 + \sigma_e^2) \quad (\text{Simm, 1998})$$

Repeatabilities (R) were calculated as the proportion of total phenotypic variation (σ_p^2) in a trait which is explained by the combined effects of additive genetic variation (σ_a^2) and permanent environmental variation (σ_{pe}^2):

$$R = (\sigma_a^2 + \sigma_{pe}^2) / (\sigma_p^2) = (\sigma_a^2 + \sigma_{pe}^2) / (\sigma_a^2 + \sigma_{pe}^2 + \sigma_e^2) \quad (\text{Simm, 1998})$$

4.3.4.2. *Estimation of Breeding Values*

The DMU1 and DMUAI modules of the software package DMU (Madsen and Jensen, 2008) were used to perform univariate BLUP evaluations using each of the three animal models defined in Chapter 4.3.3. This analysis provided BLUP of random effects and BLUE of fixed effects in each of the respective models. EBVs were therefore calculated for all 73,344 individuals in the Greyhound pedigree file, for each of the three performance traits: Race Time over a 480 m distance (20,934 animals with performance records), Speed and Rank over all distances (50,452 animals with performance records), based on their own and/or their relatives race performance (Appendix 1).

The current system in use by the UK Kennel Club (2014) is that EBVs take the same unit and direction as the original phenotype, with more negative EBVs being favourable for canine elbow and hip dysplasia as they indicate a lower genetic risk.

To adhere to this convention, performance measures for Race Time and Rank were not rescaled for calculation of EBVs in the present study. Lower Race Time and Rank values are favourable in racing Greyhounds, therefore lower (more negative) EBVs for Race Time and Rank would also be favourable. Similarly, a greater Speed is favourable and consequently a greater (more positive) EBV for Speed would be desired.

For each of the three performance traits, mean EBVs were calculated for each birth year (2000 to 2011) of Greyhounds with performance data, in order to calculate the genetic trends during this period.

The top 20 Greyhound sires and dams were identified, with respect to numbers of offspring produced within the data set, in order to compare their popularity as breeding animals with their additive genetic merit (EBV) for the three performance traits.

4.4. Results

4.4.1. Estimation of Genetic Parameters

The additive genetic (σ^2_a), permanent environmental (σ^2_{pe}) and residual variances (σ^2_e), and calculation of estimated heritabilities (h^2) for the performance trait Race Time (over a 480 m race distance), and the performance traits Speed and Rank (over all race distances), are displayed in Table 4.2. Estimates of h^2 were moderate-high for the performance trait Race Time (0.44), moderate for Speed (0.37), and low for rank (0.02). Repeatabilities were moderate-high for Race Time (0.56) and Speed (0.52), and low for rank (0.03).

Variance Component	Race Time	Speed	Rank
σ^2_a (SE)	0.1317 (0.0054)	0.0313 (0.0008)	0.0473 (0.0029)
σ^2_{pe} (SE)	0.0377 (0.0032)	0.0127 (0.0005)	0.0451 (0.0020)
σ^2_e (SE)	0.1332 (0.0003)	0.0413 (0.0001)	2.8113 (0.0031)
h^2 (SE)	0.4352 (0.0137)	0.3670 (0.0080)	0.0163 (0.0010)
R	0.5597	0.5157	0.0318

Table 4.2. The additive genetic variance (σ^2_a), permanent environmental variance (σ^2_{pe}), residual variance (σ^2_e), estimated heritability (h^2) and repeatability (R) for Race Time (over a 480 m race distance), Speed and Rank (over all distances), in British racing Greyhounds. The standard error (SE) is given in brackets.

4.4.2. Estimation of Breeding Values

EBVs were calculated for all 73,344 individuals in the Greyhound pedigree file, for each of the traits Race Time, Speed and Rank (Appendix 1). EBVs are expressed in the same units as the performance trait, and may be positive or negative. Consequently, negative EBVs are more favourable for the traits Race Time and Rank, in which a lesser Race Time is equivalent to running a faster race, and a lesser Rank value is desirable as a Rank of one (first) would be the race winner. In contrast, a positive EBV is more favourable for the trait Speed, in which a greater value is equivalent to greater genetic merit for speed. The EBVs varied from 3.340 (worst) to -1.090 (best) for Race Time over a distance of 480 m, and from -0.786 (worst) to 0.666 (best) for Speed and 0.491 (worst) to -0.633 (best) for Rank over all distances. Standard deviations were 0.196, 0.126 and 0.098 for Race Time, Speed and Rank EBVs, respectively.

4.4.3. Genetic Trends

The mean EBVs according to Greyhound year of birth are displayed in Figure 4.1, Figure 4.2 and Figure 4.3 for Race Time over a 480 m distance, Speed over all distances and Rank over all distances, respectively. Figure 4.1 indicates a decline in EBV and therefore a trend of genetic improvement in Race Time in the British racing Greyhound population, over a 12 year period from 2000 to 2011. There was a genetic improvement in Race Time of 0.25 s, equivalent to 0.021 s per year.

Figure 4.2 indicates an increase in EBV and therefore a trend of genetic improvement in Speed in the British racing Greyhound population, over the years 2000 to 2011. There was a genetic improvement in Speed of 0.16 m/s, equivalent to 0.013 m/s per year.

The genetic improvement in both Race Time (Figure 4.1) and Speed (Figure 4.2) occurred at a relatively steady rate during the 12 year period. Figure 4.3, however, demonstrates minimal genetic improvement in Rank over the years 2000 to 2010. There was a sudden decline in mean EBV from the year 2010 to 2011, however this may be an artefact. Dogs born in 2010 would only be at the start of their racing career and in their first few months of competitive racing during the final year (2012) of the study period (2008 to 2012), and would not have any performance records prior to this. Overall, there was a genetic improvement in Rank of 0.03 rank finish positions during the 12 year period from 2000 to 2011, equivalent to 0.0025 rank finish positions per year.

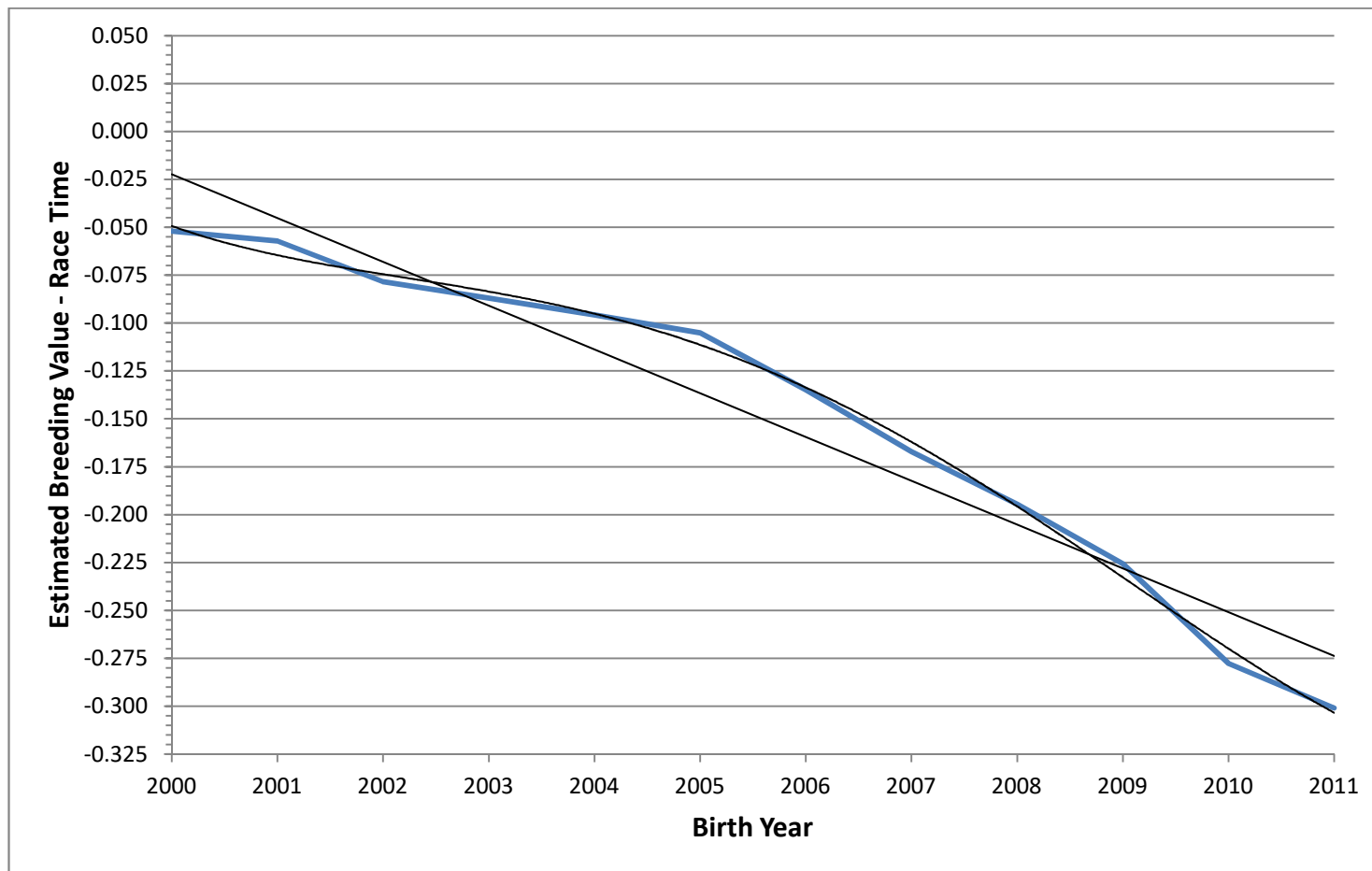


Figure 4.1. Mean Estimated Breeding Value (EBV) of British racing Greyhounds (n = 73,344) for the performance trait Race Time (over a 480 m race distance), according to birth year of the dog.

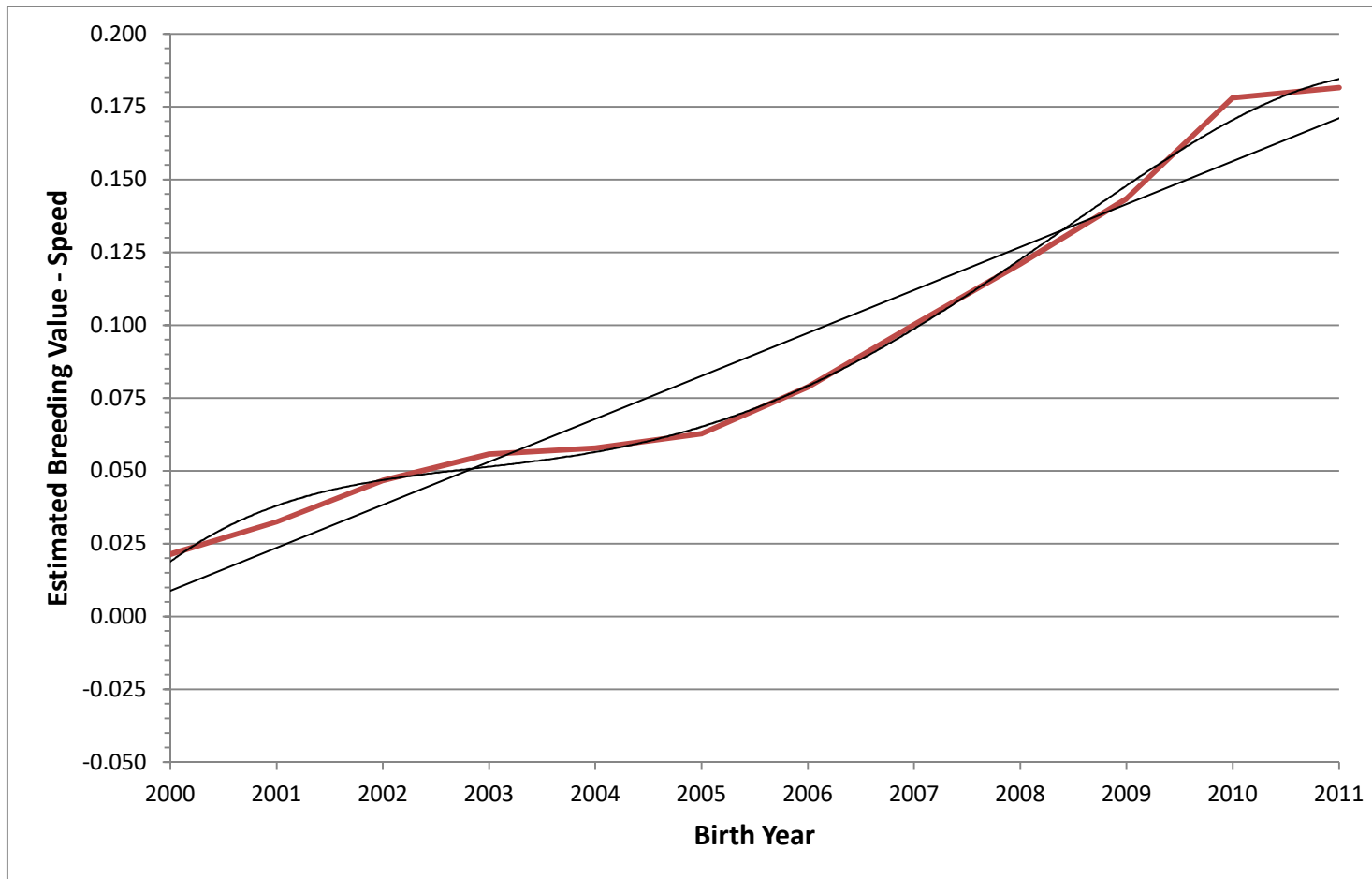


Figure 4.2. Mean Estimated Breeding Value (EBV) of British racing Greyhounds (n = 73,344) for the performance trait Speed (over all race distances), according to birth year of the dog.

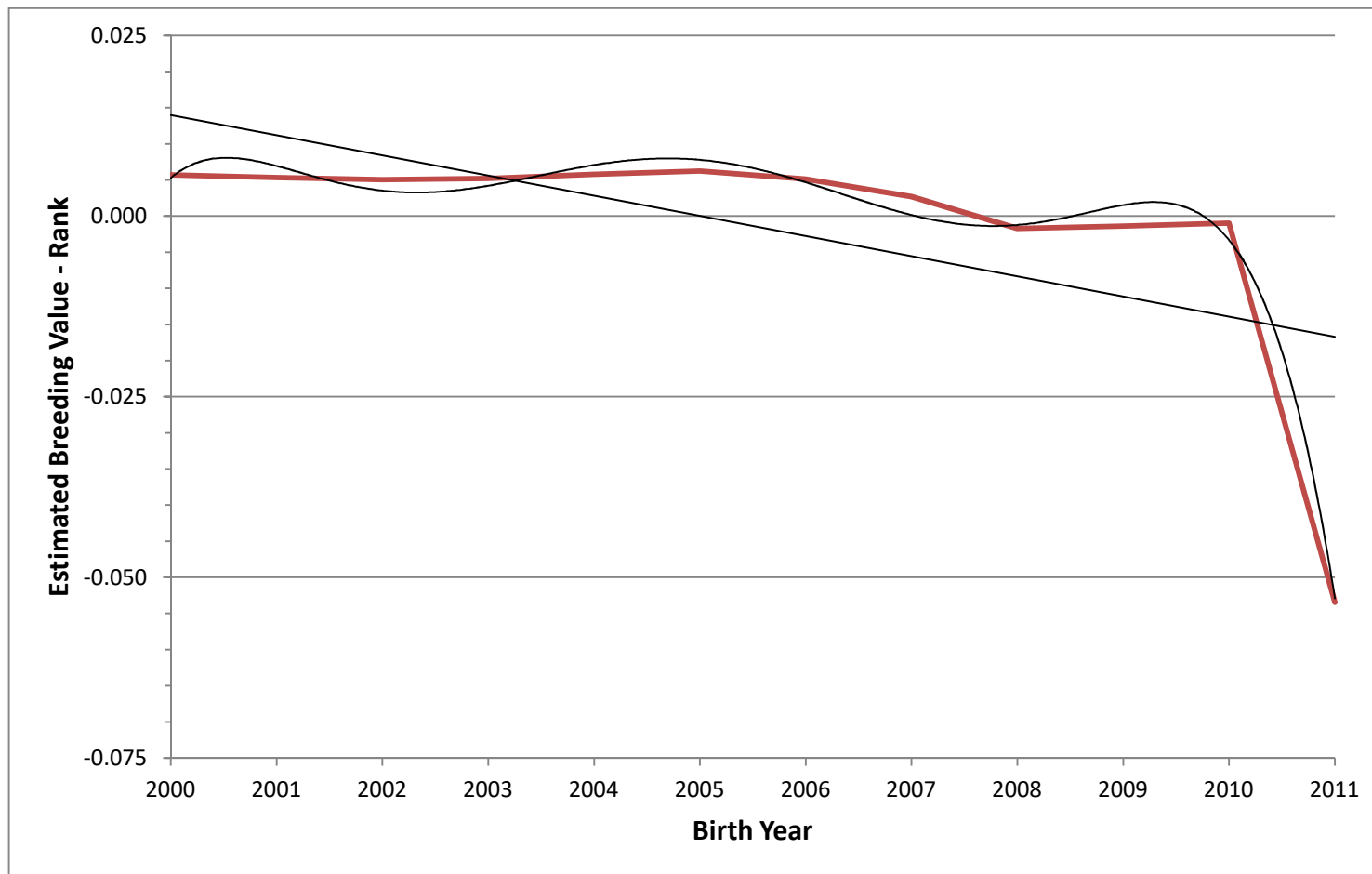


Figure 4.3. Mean Estimated Breeding Value (EBV) of British racing Greyhounds ($n = 73,344$) for the performance trait Rank (over all race distances), according to birth year of the dog.

4.4.4. EBVs of Popular Greyhound Sires

The number of offspring produced by male Greyhounds in the data set ranged from zero to 3,203. The EBVs and genetic ranking for Race Time, Speed and Rank of the top 20 Greyhound sires in the data set, with respect to numbers of offspring produced (817 to 3,203) within the data set, are displayed in Table 4.3. Considerable variation in EBVs was observed in this group of Greyhounds used most frequently for breeding. EBVs ranged from -0.031 to -0.426 (SD 0.09), 0.032 to 0.240 (SD 0.05), and 0.104 to -0.143 (SD 0.07) for the performance traits Race Time, Speed and Rank, respectively.

The EBVs for Race Time, Speed and Rank of the top 20 Greyhound sires, with respect to numbers of offspring produced, are displayed in Figure 4.4, Figure 4.5 and Figure 4.6, respectively. Interestingly, Figure 4.4 demonstrates a slight increasing trend in EBV for Race Time ($R^2 = 0.1014$) as the number of offspring produced increases. Greyhound sires with larger numbers of offspring actually have more positive (less favourable) EBVs for Race Time. Similarly, Figure 4.5 demonstrates a slight decreasing trend in EBV for Speed ($R^2 = 0.138$), and sires with greater numbers of offspring have more negative (less favourable) EBVs. There is, however, considerable variation in EBVs for both of these traits according to number of offspring produced. Figure 4.6 indicates there is no association ($R^2 = 0.0002$) between EBV for Rank and the number of offspring produced by the sire.

DogID	No. Offspring	Sire Rank	Race Time			Speed			Rank		
			EBV	SE	GR	EBV	SE	GR	EBV	SE	GR
10536	3203	1	-0.237	0.059	19650	0.148	0.025	21316	-0.076	0.037	13409
10538	3108	2	-0.183	0.060	27555	0.112	0.025	28400	-0.071	0.038	14490
10557	2704	3	-0.120	0.060	37969	0.062	0.025	39357	0.104	0.038	62145
10565	2133	4	-0.254	0.061	17678	0.127	0.025	25344	-0.079	0.039	12988
10544	2003	5	-0.287	0.060	14127	0.165	0.025	18273	-0.143	0.039	4603
10570	1983	6	-0.221	0.061	21921	0.100	0.025	30884	0.078	0.039	57306
10540	1842	7	-0.252	0.061	17890	0.142	0.025	22387	0.077	0.040	57026
10553	1807	8	-0.195	0.063	25674	0.099	0.026	31132	0.087	0.040	59109
10542	1431	9	-0.159	0.065	31626	0.118	0.026	27157	-0.052	0.042	18980
10537	1264	10	-0.234	0.065	20123	0.126	0.026	25517	-0.051	0.042	19194
10574	1192	11	-0.257	0.066	17352	0.169	0.026	17520	0.034	0.043	45853
10532	1174	12	-0.031	0.068	51987	0.032	0.027	46201	0.032	0.043	45222
10620	1090	13	-0.241	0.067	19264	0.173	0.027	16892	-0.035	0.043	23183
10590	1006	14	-0.366	0.066	8006	0.215	0.027	10965	-0.012	0.045	30099
10567	962	15	-0.358	0.067	8520	0.189	0.027	14450	0.095	0.044	60677
10605	899	16	-0.278	0.068	15079	0.161	0.027	18954	-0.062	0.046	16495
10545	873	17	-0.277	0.070	15205	0.158	0.028	19378	-0.109	0.046	8177
10626	861	18	-0.285	0.070	14377	0.161	0.028	18910	-0.022	0.045	26802
10602	826	19	-0.426	0.068	5169	0.240	0.028	8110	-0.076	0.047	13518
10549	817	20	-0.139	0.072	34769	0.089	0.028	33366	0.017	0.047	40492

Table 4.3. Estimated Breeding Values for the 20 top-ranking Greyhound sires in the data set, with respect to numbers of offspring produced, for the performance traits Race Time, Speed and Rank. *DogID* is the unique Greyhound identification number (1 - 73,344); *No. Offspring* is the number of offspring produced by the sire to date, within the data set; *Sire Rank* is the rank of the dog according to number of offspring produced; *EBV* is the calculated EBV of the dog; *SE* is the standard error of the EBV, and *GR* is the genetic rank of the dog (1 - 73,344) according to their EBV for the trait.

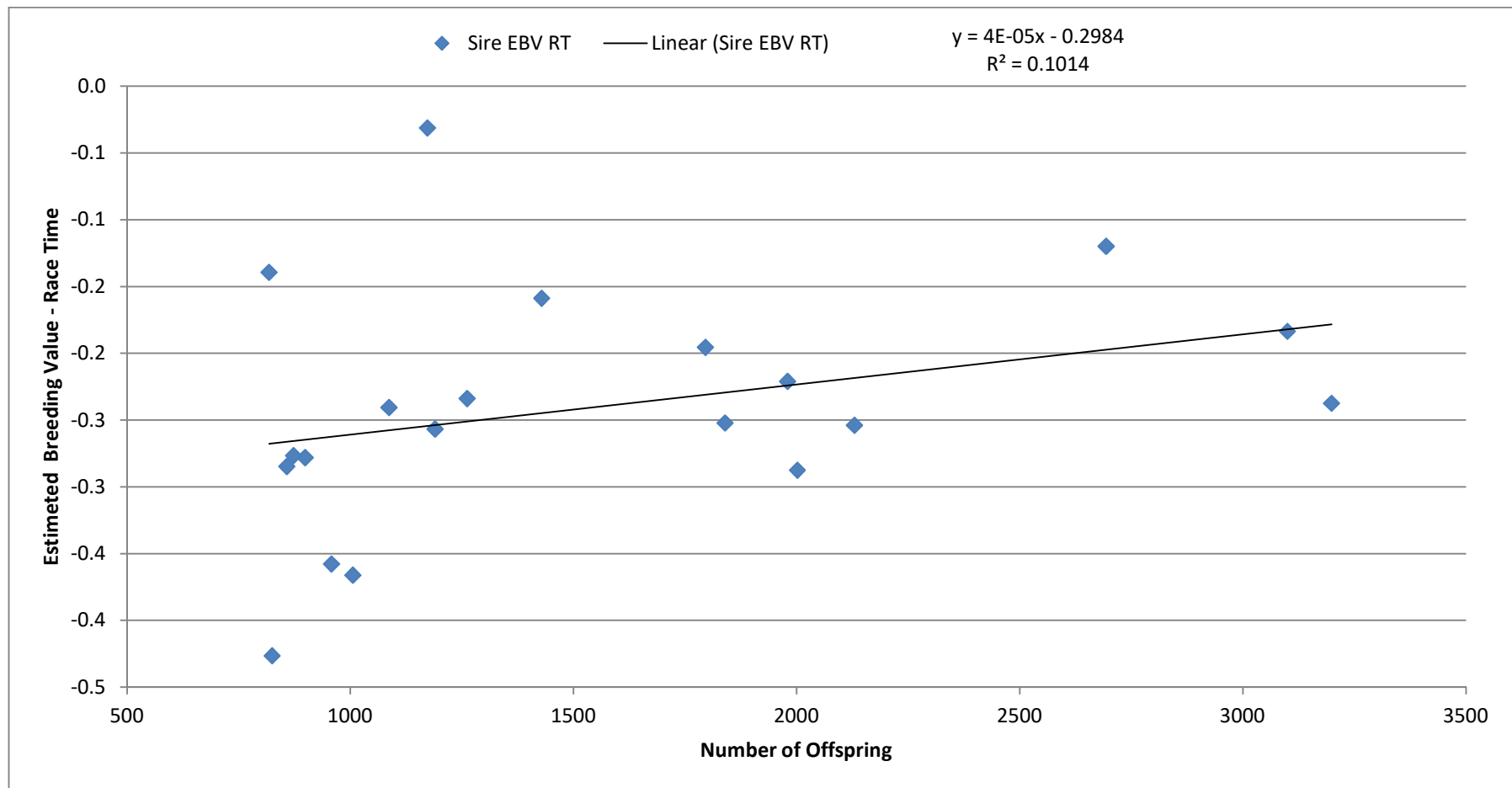


Figure 4.4. Estimated Breeding Values (EBVs) for Race Time over a 480 m race distance of the top 20 British racing Greyhound sires according to number of offspring produced.

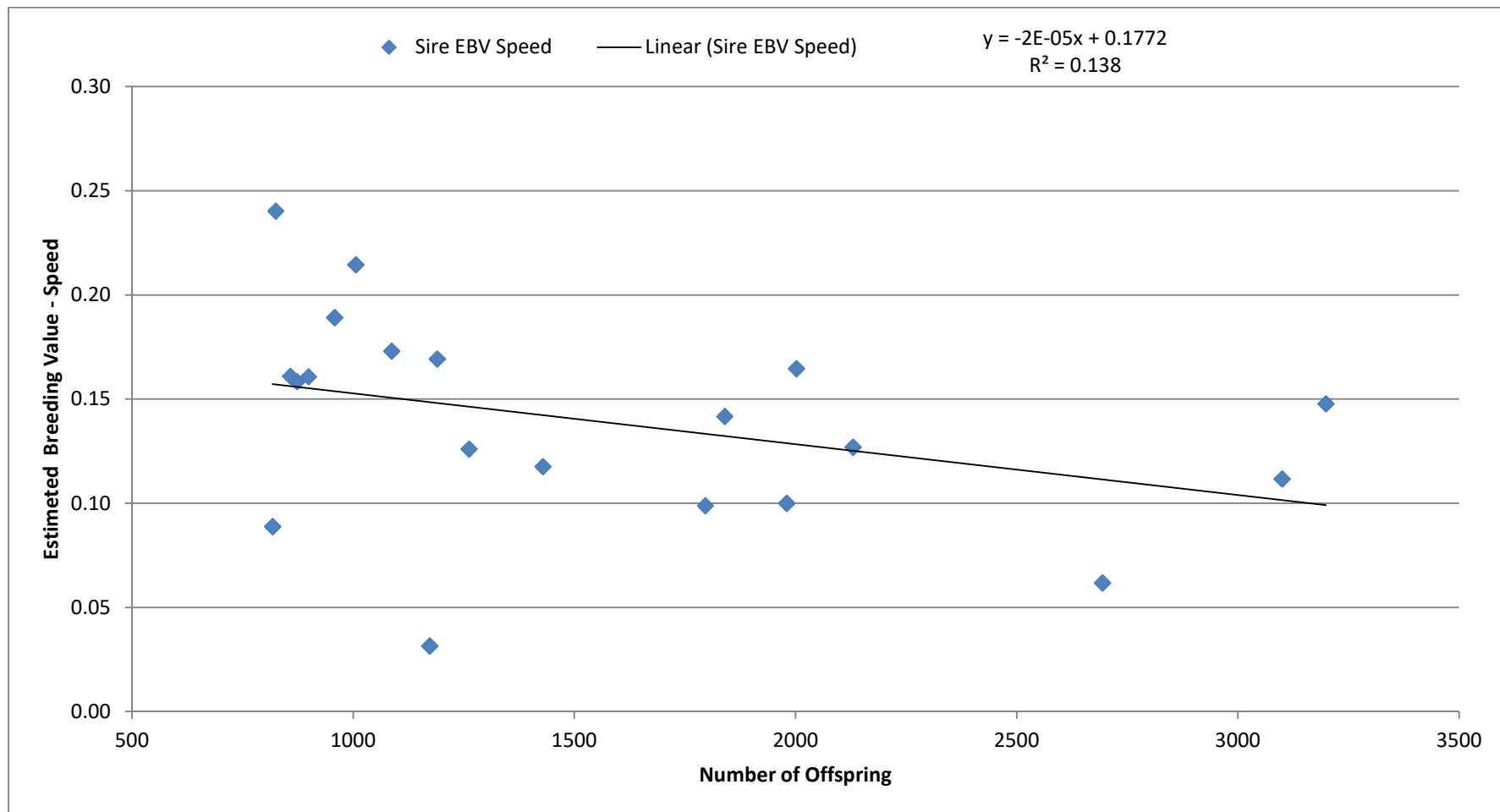


Figure 4.5. Estimated Breeding Values (EBVs) for Speed of the top 20 British racing Greyhound sires according to number of offspring produced.

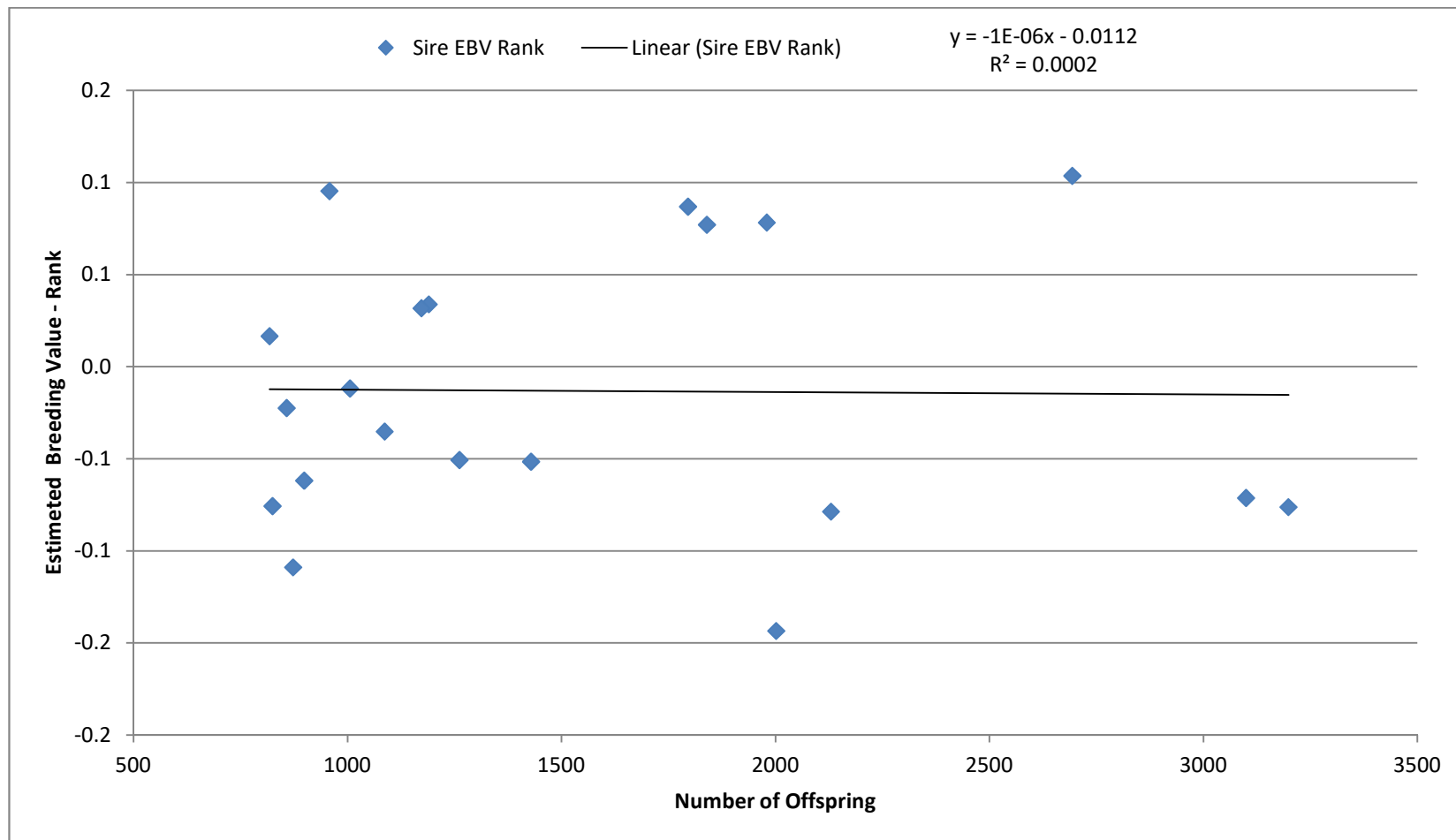


Figure 4.6. Estimated Breeding Values (EBVs) for Rank of the top 20 British racing Greyhound sires according to number of offspring produced.

4.4.5. EBVs of Popular Greyhound Dams

The number of offspring produced by female Greyhounds in the data set ranged from zero to 49. The EBVs and genetic ranking for Race Time, Speed and Rank of the top 20 Greyhound dams in the data set, with respect to numbers of offspring produced (36 to 49) within the data set, are displayed in Table 4.4. Considerable variation in EBVs was observed in this group of Greyhounds. EBVs ranged from 0.051 to -0.965 (SD 0.27), 0.035 to 0.538 (SD 0.15), and 0.392 to -0.429 (SD 0.19) for the performance traits Race Time, Speed and Rank, respectively.

The EBVs for Race Time, Speed and Rank of the top 20 British racing Greyhound dams, according to number of offspring produced, are displayed in Figure 4.7, Figure 4.8 and Figure 4.9, respectively. Figure 4.7 and Figure 4.9 indicate a slight increasing trend in EBV for Race Time ($R^2 = 0.0089$) and Rank ($R^2 = 0.0998$), respectively, as the number of offspring produced increases. Greyhound dams with larger numbers of offspring have more positive (less favourable) EBVs for Race Time and Rank. Figure 4.8 indicates a slight decreasing trend in EBV for Speed ($R^2 = 0.0686$), and dams with greater numbers of offspring have more negative (less favourable) EBVs. There is, however, considerable variation in EBVs for each of these traits according to number of offspring produced.

DogID	No. Offspring	Dam Rank	Race Time			Speed			Rank		
			EBV	SE	GR	EBV	SE	GR	EBV	SE	GR
12418	49	1	-0.668	0.121	612	0.425	0.053	396	-0.257	0.098	508
11725	47	2=	-0.556	0.159	1764	0.278	0.053	4994	-0.138	0.100	5062
14773	47	2=	-0.797	0.113	165	0.415	0.051	474	-0.176	0.086	2549
11977	46	3	-0.329	0.148	10444	0.062	0.056	39255	0.113	0.099	63625
11820	45	4	-0.452	0.163	4201	0.155	0.055	20019	0.000	0.104	34717
12499	44	5	0.051	0.158	63358	0.035	0.057	45360	0.392	0.110	73146
11048	43	6	-0.170	0.155	29733	0.105	0.056	29796	-0.111	0.103	7897
11437	42	7	-0.539	0.134	2082	0.362	0.056	1299	-0.135	0.102	5345
11774	41	8=	-0.543	0.139	2012	0.275	0.057	5228	0.087	0.112	59207
11855	41	8=	-0.965	0.156	17	0.480	0.058	120	-0.429	0.106	20
11126	40	9=	-0.076	0.159	45194	0.058	0.059	40106	0.018	0.115	40923
12717	40	9=	-0.615	0.120	1026	0.394	0.056	715	-0.009	0.101	31181
11656	39	10=	-0.227	0.161	21155	0.237	0.058	8456	-0.056	0.112	17945
15077	39	10=	-0.533	0.148	2180	0.295	0.057	3924	-0.021	0.091	27080
12510	39	10=	-0.650	0.145	762	0.458	0.059	196	-0.225	0.115	991
13586	39	10=	-0.396	0.148	6406	0.221	0.057	10174	-0.298	0.106	207
11207	38	11	-0.378	0.138	7333	0.339	0.058	1924	-0.227	0.109	955
12758	37	12	-0.922	0.127	34	0.538	0.059	25	-0.384	0.111	39
11566	36	13=	-0.811	0.141	140	0.456	0.060	202	-0.242	0.108	703
11112	36	13=	-0.289	0.176	13970	0.193	0.059	13836	-0.226	0.106	967

Table 4.4. Estimated Breeding Values for the 20 top-ranking Greyhound dams in the data set, with respect to numbers of offspring produced, for the performance traits Race Time, Speed and Rank. *DogID* is the unique Greyhound identification number (1 - 73,344); *No. Offspring* is the number of offspring produced by the dam to date, within the data set; *Dam Rank* is the rank of the dog according to number of offspring produced; *EBV* is the calculated EBV of the dog; *SE* is the standard error of the EBV, and *GR* is the genetic rank of the dog (1 - 73,344) according to their EBV for the trait.

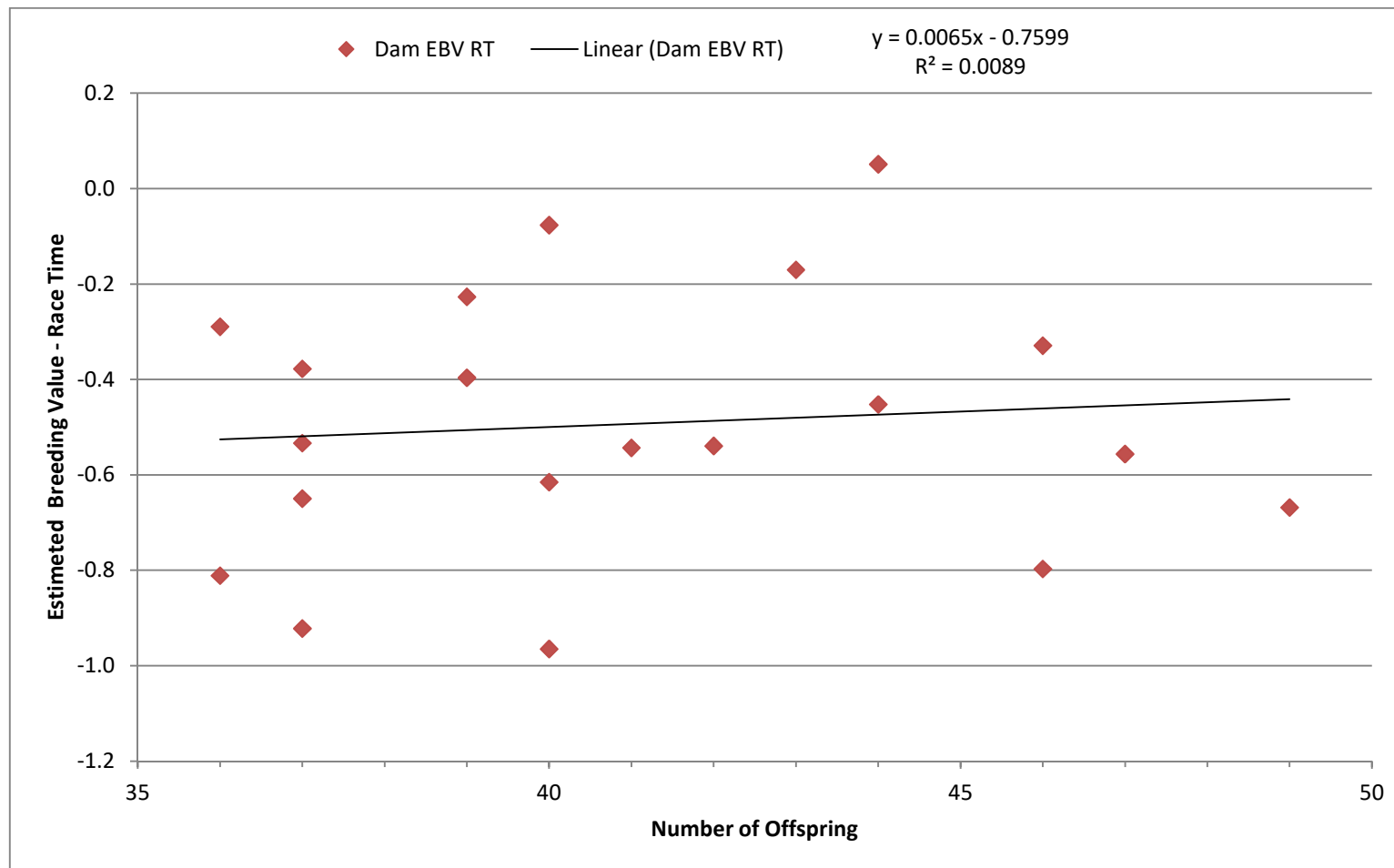


Figure 4.7. Estimated Breeding Values (EBVs) for Race Time over a 480 metre race distance of the top 20 British racing Greyhound dams according to number of offspring produced.

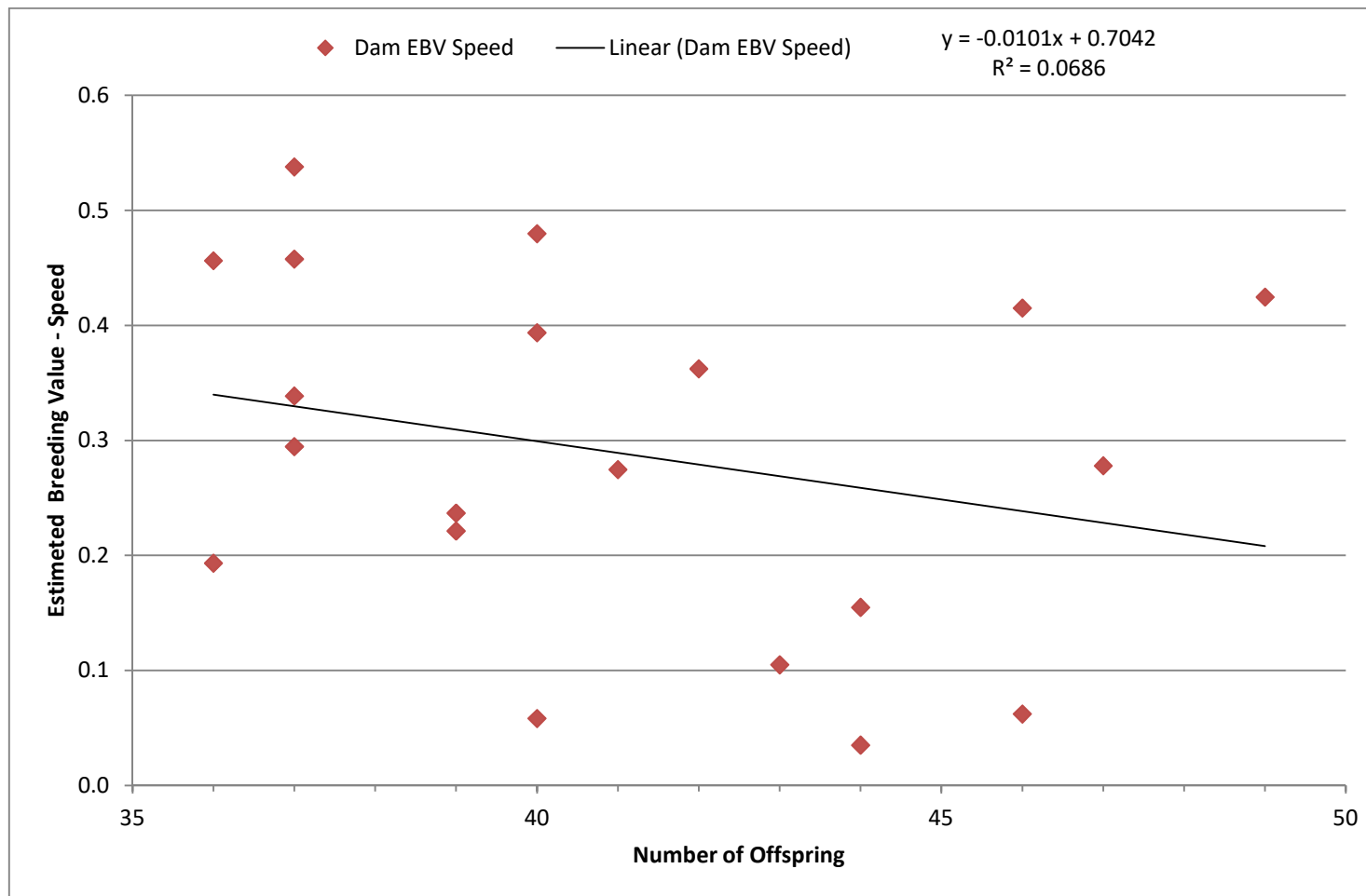


Figure 4.8. Estimated Breeding Values (EBVs) for Speed of the top 20 British racing Greyhound dams according to number of offspring produced.

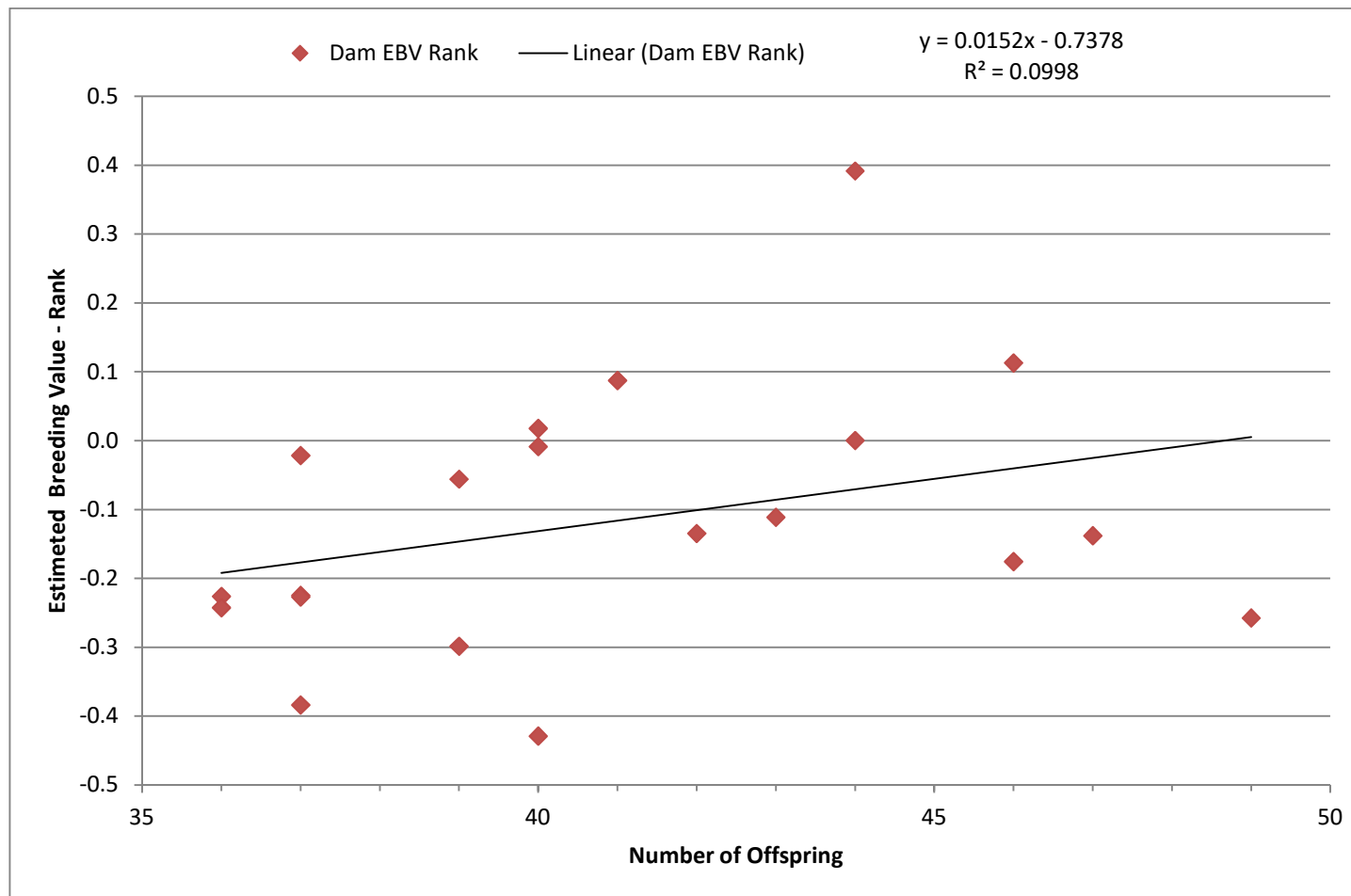


Figure 4.9. Estimated Breeding Values (EBVs) for Rank of the top 20 British racing Greyhound dams according to number of offspring produced.

4.5. Discussion

The present study is the first to investigate genetic variation in race results and estimate genetic parameters and breeding values for performance traits of British racing Greyhounds. To date, it is the largest genetic study of racing Greyhound performance worldwide. Genetic evaluations were performed on a total of 1,711,489 race performance records for 50,452 individual Greyhounds. As a result, variance components, estimated heritabilities and repeatabilities were calculated for the performance traits Race Time (over a 480 m race distance), Speed and Rank (over all race distances). For each of the three performance traits, EBVs were calculated for a total of 73,344 Greyhounds in the pedigree file.

4.5.1. Estimated Heritabilities of Greyhound Race Performance Traits

The h^2 represents the proportion of observed phenotypic variance in a trait that is due to additive genetic effects, and can therefore be passed on to the next generation. Estimated heritabilities were found to be moderate-high for Race Time (0.44), moderate for Speed (0.37) and low for Rank (0.02).

The trait of Rank at finish indicates relative performance of the Greyhound in the race. The majority of Greyhound races in the UK are graded events, in which Greyhounds compete against other dogs of the same standard with equivalent race times, to ensure each dog has a fair chance of winning and to increase the difficulty of betting. With this system, slower Greyhounds are likely to rank first as

often as faster Greyhounds. It is therefore possible, for example, that a poorer performing Greyhound running in a lower grade of race may achieve more 'first' finish positions during its career than a faster Greyhound running in races of a higher grade. A racing Greyhound's phenotypic performance in terms of Rank, however, is often a major factor taken into consideration in the selection of Greyhounds for breeding.

The heritabilities for Speed and Race Time were higher, and h^2 for Rank lower, than previously reported for racing Greyhounds (Desgorces *et al.*, 2012; Ryan, 1975; Taubert *et al.*, 2007). In a study analysing maximal speeds in horses, dogs and humans, Desgorces *et al.* (2012) compared the running speeds of the 10 best performing Greyhounds worldwide, during the period 2007 to 2009, with the individual best performance of their male ancestors over seven generations. A lower h^2 of 0.183 was reported for Speed. The study, however, analysed a limited data set considering only male ancestors, and performance results from only 67 dogs were included in the genetic analysis.

Ryan (1975) reported a lower h^2 for Race Time (0.23) in Irish Greyhounds over a race distance of 480 m. In a later study analysing performance data from 42,785 races over a 480 m distance for 42,880 Irish Greyhounds, Taubert *et al.* (2007) reported lower heritabilities of 0.31 for Race Time and 0.38 for Adjusted Race Time (a scaled logarithmic adjustment of Race Time), and a marginally higher h^2 of 0.099 for Rank. The environmental (non-genetic) factors of sex, stadium and individual race, as well as the covariate racing age, were included in the statistical mixed

model for genetic analysis. In contrast to the present study, the environmental factors of number of dogs in the race and starting trap number were not considered. This may result in an underestimation of the proportion of additive genetic variance associated with the performance traits, as several important environmental factors were not accounted for in the analysis. Additionally, pedigree data was limited to two generations for the majority of Greyhounds, with only the sire and dam of each dog with a performance result included in estimation of variance components and breeding values. In the present study, however, the maternal and paternal pedigree of each Greyhound with a race performance result was traced back six generations, resulting in comprehensive Greyhound pedigree data ($n = 73,344$ individuals) for estimation of variance components and calculation of EBVs.

The study by Taubert *et al.* (2007) included individual race event as a fixed effect in their statistical model for genetic analysis. This enables the environmental differences between race dates, race tracks, and between each race event within race tracks, to be taken into consideration. For example, environmental differences between race tracks, race dates and individual race events may include variations in geographic locations, track design, circumference and radius of the bends, track preparation, track substrate material and climatic conditions at the time of the race. Bugislaus *et al.* (2005) compared two different statistical models considering race track or individual race as a fixed effect for genetic evaluation of German trotter horses, and found increased heritabilities for performance traits, increased genetic and phenotypic correlations among traits, and a lower bias and

mean square error when including individual race rather than race track in the statistical model. Due to computational limitations as a result of the large data set analysed in the present study, it was not possible to include the effect of individual race event in the final mixed animal models for genetic analysis. Similarly, it was not possible to use a combination of stadium and race date as a fixed effect in the model. The random effect of month-year-stadium combination of the race, *MYStad*, was therefore included in the statistical models. This enables the environmental differences between race tracks, and the effects of climate and season, to be taken into consideration. It does not, however, account for variations in environmental conditions between race days in a particular month. Additionally, it does not account for environmental variation between individual race events at a particular race track on a specific date, for example due to sudden changes in weather conditions or variations in track surface conditions as the race meeting progresses.

In studies performing genetic analysis of race performance in horses, h^2 estimates reported for Race Time tend to be lower, ranging from 0.00 to 0.22 (Bakhtiari and Kashan, 2009; Buxadera and Mota, 2008; Chico, 1994; Mota *et al.*, 1998, Oki *et al.*, 1995; Taveira *et al.*, 2004; Villela *et al.*, 2002). Correa and Mota (2007), however, reported heritabilities ranging from 0.26 to 0.41 for Race Time in the Brazilian Quarter Horse. In addition, heritabilities for Race Time ranging from 0.175 to 0.304 and from 0.177 to 0.353 have been reported in Arabian horses (Ekiz *et al.*, 2005a) and Thoroughbred horses in Turkey (Ekiz and Kocak, 2007; Ekiz *et al.*, 2005b), respectively. Reported h^2 estimates for Rank tend to be higher than those

obtained in the present study, ranging from 0.04 to 0.37 and from 0.12 to 0.25 in Thoroughbred and trotter horses, respectively (Bakhtiari and Kashan, 2009; Chico, 1994; Ekiz and Kocak, 2005; Ekiz *et al.*, 2005b; Thiruvankadan *et al.*, 2009a,b; Villela *et al.*, 2002).

4.5.2. Repeatabilities of Greyhound Race Performance Traits

Repeatabilities were found to be moderate-high for Race Time (0.56) and Speed (0.52), and low for rank (0.03). These results are very similar to those reported by Taubert *et al.* (2007) for Race Time (0.56) and Adjusted Racing Time (0.51) in Irish Greyhounds, and by Meissen (1997) for Race Time (0.52) in racing whippets. Taubert *et al.* (2007), however, reported a higher repeatability for Rank (0.13). In comparison with studies of race performance in horses, Oki *et al.* (1995) and Villela *et al.* (2002) reported very similar repeatabilities for Race Time (0.55). Chico (1994), however, reported a lower repeatability for Race Time (0.01). Villela *et al.* (2002) and Chico (1994) reported higher repeatabilities for Rank of 0.44 and 0.21-0.26, respectively.

4.5.3. Estimated Breeding Values (EBVs) for British Racing Greyhounds

The present study is the first to estimate breeding values for British racing Greyhounds. EBVs were calculated for 73,344 individuals, for each of the three performance traits: Race Time (20,934 animals with performance records), Speed and Rank (50,452 animals with performance records). The EBVs varied from 3.340

(worst) to -1.090 (best) (SD 0.196) for Race Time (over a distance of 480 m), and from -0.786 (worst) to 0.666 (best) (SD 0.126) for Speed and 0.491 (worst) to -0.633 (best) (SD 0.098) for Rank (over all distances).

In the only previous study to estimate breeding values for racing Greyhounds, Taubert *et al.* (2007) calculated EBVs for 51,332 Irish Greyhounds (42,880 with performance records) for the traits Race Time and Rank (over a 480 m distance), in which performance measures were rescaled by reversing the sign to make the favourable EBVs positive. The EBVs ranged from -0.78 (worse) to 0.62 (best) (SD 0.15) for Race Time, and from -1.30 (worse) to 1.05 (best) (SD 0.25) for Rank. In order to adhere to current conventions for EBV calculation in UK dogs (The Kennel Club, 2014), performance measures were not rescaled in the present study (Chapter 4.3.4.2).

The absolute value of an EBV is not important. It is the comparison of EBVs between individuals in a specific population group that enables the individuals to be ranked in order of their additive genetic merit. In the present study, lower (more negative) EBVs are favourable for the performance traits Race Time and Rank, and greater (more positive) EBVs are favourable for Speed. The calculated EBVs are not comparable with those reported in the previous study of Irish Greyhounds (Taubert *et al.*, 2007), as EBVs are specific to a population group. Consequently, EBVs can only be used to compare the genetic merit of individuals included within the same genetic analysis.

4.5.4. Phenotypic and Genetic Trends in Greyhound Race Performance

The phenotypic development of the performance traits Race Time (over a 480 m distance) and Speed (over all distances) during the five-year study period from 2008 to 2012 are displayed in Figure 3.12 and Figure 3.13, respectively. There was a phenotypic improvement of 0.24 s in Race Time of British Greyhounds during the five-year period, and a phenotypic improvement of 0.09 m/s in racing Speed. This is equivalent to an improvement of 0.048 seconds per year in Race Time and 0.018 metres per second per year in Speed. It is not possible to attain a phenotypic improvement in Rank within the population, as a Rank of one (first) is always the highest.

In the British racing Greyhound population, there was a genetic improvement of 0.25 s in Race Time over a 12 year period from 2000 to 2011 (Figure 4.1), equivalent to a genetic improvement of 0.021 s per year. A similar genetic improvement of 0.03 s per year was reported by Taubert *et al.* (2007) for Race Time in Irish Greyhounds. For British Greyhounds, there was a genetic improvement in Speed of 0.16 m/s over a 12 year period from 2000 to 2011 (Figure 4.2), equivalent to a genetic improvement of 0.013 m/s per year. In addition, there was an overall genetic improvement of 0.03 Rank finish positions in British Greyhounds over the 12 year period (Figure 4.3), equivalent to a genetic improvement of 0.0025 Rank finish positions per year. The genetic improvement in Rank over time is more difficult to interpret, as a phenotypic improvement is not

possible. If individuals with better (lower) Rank finish positions are more likely to be selected as parents, then genetic improvement in Rank may be possible over time as a small proportion of the phenotypic variation in Rank was found to be a result of additive genetic effects ($h^2 = 0.02$). Alternatively, the observed genetic improvement in Rank over time may have been due to genetic improvement in a different trait that is genetically correlated to Rank. Taubert *et al.* (2007) reported no genetic improvement in Rank of Irish Greyhounds from the years 1994 to 2002, when analysis was performed using a single trait model. A positive genetic improvement in Rank was, however, obtained for the bivariate model, and this may have been due to the high genetic correlation between Race Time and Rank (Taubert *et al.*, 2007).

4.5.5. EBVs of Prolific British Greyhounds

The current method of Greyhound selection for breeding is based on their own or their relative's phenotypic racing performance. This traditional approach to selective breeding usually involves selection of dogs, or relatives of dogs, that have performed well in terms of race finish times or number of races won (Amphlett, 1993; Clarke, 1980; Genders, 1975). Breeding using AI is common, resulting in the most popular Greyhound sires producing very large numbers of offspring.

Defining a Greyhound sire or dam as a dog with at least one progeny in the data set, there were a total of 2,820 sires and 18,507 dams out of a total of 50,452 British Greyhounds with performance records over a five-year period from 2008 to

2012. In comparison, Taubert *et al.* (2007) reported a total of 793 sires and 8,136 dams out of a population of 42,880 Irish racing Greyhounds over a four-year period from 2000 to 2003.

With the increased use of AI, relative ease of transport of semen between countries and ability to store canine semen long-term, it is possible for Greyhound sires to produce offspring worldwide and to continue to do so for many years after their death. This has resulted in the most popular racing Greyhound sires producing thousands of offspring per sire. In a study of 42,880 Irish Greyhounds, Taubert *et al.* (2007) reported that 43.7% of the total number of offspring in the study population had descended from just 18 Greyhound sires used extensively for breeding, each with over 500 progeny.

4.5.5.1. *EBVs of Prolific Greyhound Sires*

In the top 20 Greyhound sires in the present study, with respect to numbers of progeny in the data set, numbers of offspring produced per sire ranged from 817 to 3,203. There was considerable variation in EBVs for Race Time (-0.031 to -0.426), Speed (0.032 to 0.240) and Rank (0.104 to -0.143) within this group of Greyhounds used most frequently for breeding (Table 4.3). Within the top 20 Greyhound sires, all EBVs were negative for Race Time and positive for Speed. None of these dogs, however, ranked in the top 5,000 Greyhounds overall in terms of genetic merit for the moderate-highly heritable trait Race Time ($h^2 = 0.44$) or the moderately heritable trait Speed ($h^2 = 0.37$). Three dogs (*DogID* 10,602,

Genetic Rank 5,169; *DogID* 10,590, *Genetic Rank* 8,006 and *DogID* 10,567, *Genetic Rank* 8,520) ranked in the top 10,000 Greyhounds with respect to genetic merit for Race Time. There was only one dog (*DogID* 10,602, *Genetic Rank* 8,118) ranked in the top 10,000 Greyhounds for Speed, and only a further eight dogs ranked within the top 20,000 Greyhounds. The most prolific breeding sire (*DogID* 10536) in the data set, with 3,203 progeny produced to date, was actually ranked 19,650th and 21,316th overall in terms of genetic merit for Race Time and Speed, respectively.

Taubert *et al.* (2007) similarly reported considerable variation in EBVs for the 10 Irish Greyhound sires with the highest number of offspring (851 to 2,031 progeny), with EBVs ranging from 0.45 to -0.01 for the trait Racing Time. The 10 Greyhound sires used most intensively for breeding in Ireland were not the dogs with the best EBVs. Only one of the 10 dogs with the highest number of offspring had an EBV for Race Time among the top 30 evaluated Greyhounds. The dog with the eighth highest number of offspring (991 progeny) actually had a negative (less favourable) EBV of -0.01.

4.5.5.2. *EBVs of Prolific Greyhound Dams*

In the top 20 Greyhound dams in the present study, with respect to numbers of progeny in the data set, numbers of offspring produced per dam ranged from 36 to 49. There was considerable variation in EBVs for Race Time (0.051 to -0.965), Speed (0.035 to 0.538) and Rank (0.392 to -0.429) within this group of Greyhounds (Table 4.4). In contrast to the top 20 breeding sires, however, two of the dams (*DogID*

11855, *Genetic Rank* 17 and *DogID* 12758, *Genetic Rank* 34) were ranked in the top 50 Greyhounds overall in terms of genetic merit for Race Time, and a further two dams were ranked within the top 500 Greyhounds. One of these dogs (*DogID* 12758, *Genetic Rank* 25) was also ranked in the top 50 Greyhounds overall in terms of genetic merit for Speed, and a further five dams were ranked within the top 500 Greyhounds.

There is a physiological limit on the numbers of litters and puppies a Greyhound bitch can produce during her lifetime. Additionally, the Breeding and Sale of Dogs (Welfare) Act 1999, which applies to all breeding establishments producing dogs for sale, specifies that bitches cannot be mated before they are 12 months of age, can only produce one litter every 12 months, and must produce no more than six litters in a lifetime. Consequently, the number of offspring produced per Greyhound dam is usually considerably less than the number produced per Greyhound sire.

4.5.6. Selection of Greyhounds for Breeding

Moderate to high heritabilities and a trend of both phenotypic and genetic improvement over time in the performance traits Race Time and Speed, as well as a trend of genetic improvement in Rank, indicate that the current selection method for breeding based on phenotypic performance is effective for Race Time, Speed and Rank in British Greyhounds. This is, however, a slow and inefficient

method of animal improvement, and is not optimal for achieving genetic gain within the racing Greyhound population (Taubert *et al.*, 2007).

The calculation of Race Time, Speed and Rank EBVs for 73,344 British Greyhounds has identified that the dogs used most frequently for breeding, particularly the Greyhound sires producing large numbers of offspring, are not those with the greatest genetic merit. A major welfare concern in the Greyhound racing industry is the current over-breeding and production of surplus young Greyhounds that do not make the grade to race. It is likely that the main factor in this over-production and wastage is the inefficient selection of Greyhounds for breeding, with young dogs subsequently not performing as expected or as required.

The results of the present study will potentially impact the Greyhound racing industry enormously, by demonstrating the urgent requirement for a move from breeding based on traditional methods of selection towards scientific methods using quantitative genetic analysis. Targeted genetic selection based on EBVs would be considerably more efficient, therefore resulting in less wastage and a faster rate of genetic improvement within the population compared to breeding based on phenotypic selection alone. This is likely to result in economic benefits across the entire Greyhound industry, for breeders, owners, trainers, regulatory and funding bodies, rehoming centres and rescue organisations. Importantly, targeted selection based on genetic merit may improve Greyhound welfare by enabling breeders to produce Greyhounds of predictable and superior genetic

merit, therefore significantly reducing the current issue of over-breeding and wastage of young dogs in the industry that do not make the grade to race.

4.5.7. Future Work

Genetic evaluations and calculation of EBVs for specific traits are dependent on the availability of accurate phenotypic and performance data relating to those traits. Should suitable data be available, further work could be performed to calculate variance components, estimated heritabilities, repeatabilities and EBVs for additional Greyhound performance, health and welfare-related traits.

A potential negative impact of selecting Greyhounds for breeding based solely on race time or speed is that by increasing the speed of the Greyhound population we may in fact be selecting for dogs with lighter and weaker bones, and consequently dogs with a higher risk of injury and bone fracture. Several studies have reported increased rates of injuries in British Greyhounds as track running conditions become faster (Iddon *et al.*, 2014; Prole, 1976). Additionally, in a survey of orthopaedic injuries at five Greyhound tracks in Wisconsin, USA, the rate of injury increased significantly with increasing grade of race, suggesting a possible correlation between injury rate and racing speed (Sicard *et al.*, 1999).

Historically, Greyhound injury data has been routinely collected by the RCPA for many GBGB-licensed race tracks in the UK. This injury data, however, was not usually made available, even on an anonymous basis, to anyone except a few

individuals within the RCPA and the Greyhound industry regulatory bodies (APGAW, 2007). At the time of the study, there was no accessible central database of injury data for racing Greyhounds in the UK. Currently, injury data and statistics are never published, neither for individual tracks nor the British Greyhound industry as a whole, even on an anonymous basis or in summary form. Consequently, it was not possible to consider quantitative genetic analysis of injury-related traits in the present study.

EBVs are dynamic and can change when additional phenotypic, performance or pedigree data becomes available for a dog. In recent years, the GBGB have developed a Track Injury Database to act as a central repository for injury data collected from each of the GBGB-licensed stadiums (Greyhound Board of Great Britain, 2015). Should injury data become accessible in the future, it would therefore be possible to incorporate this in the genetic evaluations of racing Greyhounds and produce EBVs for specific health and welfare-related traits, such as bone fracture risk, trainability and longevity. It would then be possible for EBVs for speed and injury risk, for example, to be combined in a selection index. This would enable breeders and owners to select Greyhounds for breeding based on genetic merit for both speed and injury-resistance combined, therefore resulting in genetic improvement in the two traits simultaneously within the population. Similarly, genetic evaluations could be performed and EBVs calculated for other Greyhound health, welfare and economically-important traits where recorded data is available. The EBVs produced could be combined and used to create a

selection index that aids in selection of stronger, healthier, less injury-prone and higher-performing dogs of predictable and superior genetic merit.

Such methods have been very successfully applied to other groups of animals for the simultaneous genetic improvement of a number of industry-important traits (Simm, 1998). Signet (2015) currently offer five breed-specific selection indexes for the genetic improvement of British beef cattle: the Beef Value Index based on EBVs for birth weight, 400-day growth, muscling score, muscle depth and back fat depth; the Calving Value Index combining EBVs for gestation length and calving ease; the Maternal Value Index using EBVs for longevity, age at first calving, 200-day milk, maternal calving ease and calving interval; the Maintenance Value Index based on EBVs for cow weight; and the overall Maternal Production Value Index calculated from the other four indexes. Each trait is weighted within the particular index according to its economic importance in meeting specific breeding criteria and objectives. Signet (2015) also offer five selection indexes for British sheep. Similarly, the national Profitable Lifetime Index (£PLI) has been successfully used as a breed-specific screening tool for bull selection in British dairy herds, in order to improve overall profitability (Agriculture and Horticulture Development Board, 2014). The index combines EBVs for milk yield, milk quality, conformation, longevity, maintenance costs, fertility and calving ease.

In addition, approximately 75% of Greyhounds that race in the UK were originally bred and born in Ireland, with the remaining 25% bred in Britain (APGAW, 2007). Consequently, the majority of Greyhounds in the data set of the present study

were originally bred in Ireland, before being transferred to the UK at an early stage in their racing career. Further work may involve collaboration with the Irish Greyhound racing authorities, to enable EBVs to be made available to breeders and owners in Ireland where the majority of Greyhounds for the British racing industry are currently produced.

4.5.8. Conclusions

In the largest genetic study of racing Greyhound performance to date and the first to calculate variance components and EBVs for British Greyhounds, a total of 1,711,489 race results for 50,452 individual dogs were examined. In conclusion, estimated heritabilities were found to be moderate-high for the trait Race Time (0.44), moderate for Speed (0.37) and low for Rank (0.02). Repeatabilities were moderate-high for Race Time (0.56) and Speed (0.52), and low for rank (0.03). EBVs were calculated for 73,344 British Greyhounds, and varied from 3.340 (worst) to -1.090 (best) (SD 0.196) for Race Time, -0.786 (worst) to 0.666 (best) (SD 0.126) for Speed and 0.491 (worst) to -0.633 (best) (SD 0.098) for Rank. Over a 12 year period, genetic improvements equivalent to 0.021 s per year, 0.013 m/s per year and 0.0025 rank finish positions per year were found for the performance traits Race Time, Speed and Rank, respectively. There was, however, considerable variation in EBVs for Race Time (-0.031 to -0.426) (SD 0.09), Speed (0.032 to 0.240) (SD 0.05) and Rank (0.104 to -0.143) (SD 0.07) within the group of 20 Greyhounds with the greatest numbers of offspring (817 to 3,203 progeny). For the performance traits examined, the British Greyhound sires used most frequently for

breeding were not those with the greatest genetic merit. Using speed (or race time as a function of speed) as an example trait, the present study demonstrates that targeted genetic selection based on EBVs would be considerably more efficient than the current selection method of breeding based on phenotypic performance. EBV-based selection for a combination of health, welfare and economically important traits can be used to produce dogs of predictable and superior genetic merit. Consequently, this may improve Greyhound welfare considerably by minimising the number of surplus dogs produced.

Chapter Five.

Genome-Wide Association Study of Stress Fracture Injuries in the Racing Greyhound

5. Genome-Wide Association Study of Stress Fracture Injuries in the Racing Greyhound

5.1. Introduction

5.1.1. Stress Fractures

5.1.1.1. *Stress Fracture Injuries of the Racing Greyhound*

Racing Greyhounds sustain several specific musculoskeletal injuries that are relatively uncommon in other working or companion dogs (Davis, 1967; Hickman, 1975; Prole, 1976; Vaughan, 1969). This is a result of both their anatomy and the nature of repetitive high-speed racing in an anticlockwise direction around elliptical tracks (Davis, 1973; Prole, 1976). A combination of environmental factors are likely to contribute to the occurrence of racing injuries, including sex of the dog; fitness level; grade of race; speed; race distance; track design; degree of banking and radius of the bends; track surface material and conditions; track maintenance; and the weather (Cook, 1998; Davis, 1973; Gannon, 1972; Hickman, 1975; Sicard *et al.*, 1999; Prole, 1976; Vaughan, 1969). The influence of genetic factors on the occurrence of racing injuries in Greyhounds, however, has not previously been reported.

Bone fractures are common racing injuries of Greyhounds (Prole, 1976; Sicard *et al.*, 1999), and usually occur in the distal limb (Gannon, 1972). The majority of fractures are not associated with direct trauma (Siccard *et al.*, 1999), but are

instead considered a result of bone fatigue, due to cyclic compressive loading causing an accumulation of micro-damage in the bone beyond its rate of repair by remodelling. Catastrophic fracture eventually occurs through micro-crack dissemination or coalescence. Such fractures are known as fatigue or stress fractures (Devas, 1961; Gannon, 1972; Taylor 1997, 1998).

In the racing Greyhound, stress fractures have been described in the CTB (Bergh *et al.*, 2012; Boudrieau *et al.*, 1984a, 1984b; Devas, 1961; Emmerson *et al.*, 2000; Gannon, 1972; Johnson *et al.*, 2000; Muir *et al.*, 1999; Tomlin *et al.*, 2000), the MC and MT bones (Bellenger *et al.*, 1981; Emmerson *et al.*, 2000; Gannon, 1972; Johnson *et al.*, 2001; Lipscomb *et al.*, 2001) and the acetabulum (Wendelburg *et al.*, 1988). Stress fractures are frequently observed in human athletes (Brukner *et al.*, 1996; Devas, 1969), racehorses (O'Sullivan and Lumsden, 2002, 2003) and in military recruits (Cline *et al.*, 1998; Kowal, 1980; Ross and Allsopp, 2002). Stress fractures are common injuries of the racing Greyhound, however they are rarely seen in other breeds of dog (Emmerson *et al.*, 2000).

Stress fractures of the tarsal bones (Figure 1.4 and Figure 1.5) are one of the most common catastrophic and career-threatening injuries of the racing Greyhound, and account for approximately 67% of all injuries to the tarsus in British Greyhounds (Prole, 1976). The majority of these injuries involve fracture of the CTB (Boudrieau *et al.*, 1984a; Gannon, 1972; Guilliard, 2000; Prole, 1976), and most frequently occur in the right pelvic limb (Boudrieau *et al.*, 1984a; Davis, 1967; Devas, 1961; Gannon, 1972; Guilliard, 2000; Keene and Yarborough, 1966; Prole,

1976). This is most likely due to the stresses placed on the right tarsal joint during anticlockwise racing at high speeds (Anderson *et al.*, 1995). In the UK, Greyhounds sustaining tarsal fractures are often retired from racing or euthanased, mainly due to the financial costs of surgical treatment, the requirement for a long rest period and the possibility that the Greyhound may not regain its previous racing performance (Guilliard, 2000; Jones, 2009).

In addition, stress fractures of the MC (Figure 1.2 and Figure 1.6) and MT (Figure 1.3 and Figure 1.5) bones are frequently observed injuries of the racing Greyhound (Bellenger *et al.*, 1981; Emmerson *et al.*, 2000; Gannon, 1972; Johnson *et al.*, 2001; Lipscomb *et al.*, 2001; Piras, 2005). The majority of MC fractures occur in the left thoracic limb, with the fifth metacarpal bone (MC5) most frequently affected. This is thought to be the result of increased loading of the MC bones closest to the inner circumference of the track during anticlockwise racing (Bellenger *et al.*, 1981; Emmerson *et al.*, 2000; Gannon, 1972; Prole, 1967). Stress fractures of the left fourth (MC4) and the right second (MC2) and third (MC3) metacarpal bones are also commonly observed (Bellenger *et al.*, 1981; Gannon, 1972; Piras, 2005). In the pelvic limb, stress fractures of the left fifth (MT5) and the right third (MT3) metatarsal bones are most frequent (Piras, 2005).

5.1.1.2. *Genetic and Environmental Risk Factors for Stress Fracture Injuries*

It is hypothesised that stress fracture is a multifactorial multigenic disorder resulting from the combined effects of both genetic and environmental factors

(Yanovich *et al.*, 2012). A number of risk factors have been identified in the development of stress fractures in human athletes and military recruits, including training intensity, tibia width, training surface, nutrition, smoking and motivation, gender, age, height, type of physical activity, fitness level at the start of a physical training program, serum parathyroid hormone concentration, serum vitamin D concentration, and BMD (Jones *et al.*, 2002; McClung and Karl, 2010; Ruohola *et al.*, 2006; Valimaki *et al.*, 2005a).

There is indirect evidence suggesting the involvement of genetic factors in the pathogenesis of stress fractures in humans (Friedl *et al.*, 1992; Giladi *et al.*, 1986; Givon *et al.*, 2000; Milgrom *et al.*, 1985; Singer *et al.*, 1990). In addition, several candidate gene association studies have been performed to investigate genetic factors in the development of stress fractures in military recruits (Chatzipapas *et al.*, 2009; Korvala *et al.*, 2010; McClung and Karl, 2010; Valimaki *et al.*, 2005b; Yanovich *et al.*, 2011, 2012). Chatzipapas *et al.* (2009) reported that the *FokI* and *BsmI* polymorphisms in the VDR gene were independent risk factors for stress fracture in Greek military recruits, associated with a 2.7-fold and 2.0-fold increased risk, respectively.

The investigation of genetic factors in the pathogenesis of stress fractures in racing Greyhounds, however, has not previously been reported.

5.1.2. Genome-Wide Association Studies (GWAS)

Sequencing of the canine genome in 2005 led to the development of an extensive canine SNP map, containing 2.5 million SNPs with even distribution across the canine genome and high cross-breed polymorphism (Lindblad-Toh *et al.*, 2005). Consequently, the production of canine whole-genome SNP genotyping arrays has enabled the use of GWAS to investigate polymorphisms associated with disease in any breed of dog (Baird *et al.*, 2014; French *et al.*, 2012; Massey *et al.*, 2014; Tsai *et al.*, 2011; Wood *et al.*, 2009; Zhao *et al.*, 2014). GWA studies use an unbiased hypothesis-free approach to investigate genetic variations associated with a particular phenotype, by examining known SNPs across the entire genome in a case-control study design. Genotypes of affected (case) and unaffected (control) individuals are compared, in order to detect differences in the DNA sequence and therefore identify regions of chromosomes that may be associated with the trait. GWAS are particularly useful for detecting genetic variations that contribute to common, complex diseases, and have become the method of choice for such studies (Iles, 2011).

Within-breed GWAS can identify regions of chromosomes associated with the disease, which are then validated using fine-mapping of a larger cohort from the same population. This is followed by targeted sequencing across the region to identify candidate causal variants (Bokyo, 2011).

5.1.2.1. *Linkage Disequilibrium*

The number of SNPs required for GWAS varies according to the pattern of linkage disequilibrium (LD) within the population (Andersson, 2009). LD is defined as the non-random association of alleles at two or more loci, such that certain combinations of alleles are more likely to occur together on a chromosome than others. The human population is out-bred and genetically diverse, with LD extending over relatively short distances of 10 to 100 kilobases (kb) (Reich *et al.*, 2001). In comparison, canine breeds are of recent origin, with population bottlenecks, strict breeding rules and use of popular sires creating genetically isolated populations, with reduced genetic diversity within breeds. This has resulted in large haplotype blocks and extensive within-breed LD (0.5 - 1.0 megabases), extending up to 100 times further than in humans (Lindblad-Toh *et al.*, 2005; Sutter *et al.*, 2004). Consequently, fewer genetic markers (approximately 15,000 to 30,000 SNPs) are required for GWAS to detect disease associations in dogs, compared to an estimated 500,000 SNPs in humans (Lindblad-Toh *et al.*, 2005; Sutter *et al.*, 2004).

5.1.2.2. *GWAS Sample Sizes*

Large sample sizes (usually multiple thousands) are required for human GWAS, to ensure sufficient statistical power to detect an associated locus. The criteria for case and control selection, logistics of DNA sample collection and current costs of genome-wide SNP genotyping can restrict the practicable sample size for canine GWA studies. As a result of canine breed structure, small breeding populations and

the use of popular sires exaggerating the genetic contribution of a single dog to the whole breed, however, canine traits can be successfully mapped using relatively small sample sizes (Parker, 2012). Karlsson *et al.* (2007) demonstrated that using 27,000 SNPs, a Mendelian trait can be mapped using approximately 10 affected (case) and 10 unaffected (control) dogs. Power calculations have indicated that for a multigenic trait, a study using 100 affected and 100 unaffected dogs would have 97% power or 50% power to detect an allele that increases risk by a multiplicative factor (λ) of five or two, respectively (Lindblad-Toh *et al.*, 2005). Many canine GWAS, however, have identified statistically significant genetic risk factors associated with disease using smaller sample sizes than this.

Wilbe *et al.* (2010) identified five loci associated with a canine systemic lupus erythematosus related disease complex by conducting a GWAS with 81 affected and 57 unaffected dogs. The authors concluded that the homogeneity of strong genetic risk factors within canine breeds enables multigenic diseases to be mapped with fewer than 100 affected and 100 unaffected dogs. Wood *et al.* (2009) identified SNPs associated with canine atopic dermatitis by conducting a GWAS using 25 affected and 23 unaffected dogs. Similar numbers of cases and controls were used in a discovery GWAS to identify regions nominally associated with anal furunculosis in the German shepherd dog (Massey *et al.*, 2014). Consequently, an initial case-control discovery GWAS can be performed using a small number of affected and unaffected dogs, to identify regions of chromosomes associated with stress fracture in the racing Greyhound. Any associations identified would require confirmation by fine-mapping in a validation study, and subsequent targeted

sequencing of the identified regions of interest, in order to determine specific genetic risk factors.

5.2. Aims

The aims of the study were to establish the first DNA archive for British racing Greyhounds, for use in the present study and for future scientific research, and to perform a discovery GWA study investigating genetic factors that may predispose racing Greyhounds to developing stress fracture injuries of the distal limb.

5.3. Materials and Methods

5.3.1. Sample Collection and Preparation

A total of 237 canine DNA samples were collected from case (affected) racing Greyhounds that had taken part in less than 50 races and had previously sustained a tarsal, metacarpal or metatarsal stress fracture (n = 91 dogs), and from healthy control (unaffected) racing Greyhounds that had competed in over 70 races and never sustained a fracture or other orthopaedic injury (n = 146 dogs), as described in Chapter 2.2.1.

All control Greyhound DNA samples were collected using Oragene®-Animal OA-400 saliva collection kits (Oragene®, DNA Genotek Inc., Ontario, Canada). DNA

samples from case Greyhounds were obtained from either saliva samples collected using Oragene®-Animal kits, or from collection of muscle or skin tissue samples post-mortem. Muscle tissue samples were collected from 51 affected Greyhounds (33 males and 18 females), and skin samples from two affected Greyhounds (one male and one female), that had each sustained a stress fracture of the tarsus and had been euthanased at the race track for reasons unrelated to the present study. Saliva samples were collected from 38 affected Greyhounds (21 males and 17 females) that had previously sustained a stress fracture of the tarsus (30 Greyhounds), metacarpus (two Greyhounds) or metatarsus (six Greyhounds), and were returned to racing or retired. In addition, saliva samples were collected from 146 healthy control Greyhounds (80 males and 66 females). The various sample collection methods are described in Chapter 2.2.1.

Phenotypic and performance data were obtained for each Greyhound, and their pedigree traced back six generations using the online resource Greyhound-Data (2014).

5.3.2. DNA Extraction and Quantification

Extraction of DNA from the saliva, muscle and skin tissue samples was performed by the author as described in Chapter 2.2.2. For each sample, DNA was then quantified using the Quant-iT™ dsDNA Broad-Range Assay Kit (Invitrogen™, Life Technologies, Paisley, UK) and measured with a Qubit® fluorometer (Invitrogen™,

Life Technologies, Paisley, UK), according to the manufacturer's instructions. The extracted DNA samples were stored at -40°C until required for further processing.

5.3.3. Selection of Case and Control Samples for GWAS

Illumina® genome-wide SNP genotyping requires 4 µl of DNA per sample, at a recommended minimum concentration of 20 ng/µl. None of the DNA samples extracted from Greyhound muscle or skin tissue contained a sufficient concentration of DNA to be suitable for inclusion in the GWAS. As a result, all of the case and control DNA samples selected for Illumina® genotyping were samples extracted from Greyhound saliva collected using the Oragene® Animal kits.

In the present study, the logistics of a GWA study restricted the number of samples to 24 cases and 24 controls. DNA samples from 24 racing Greyhounds (13 males and 11 females) that had sustained a stress fracture of the distal limb were selected as cases for the GWA study. The injuries sustained by these Greyhounds consisted of 19 tarsal fractures (11 right limb, two left limb, one both right and left limb, and five unknown limb); four MT fractures (two left limb, one right limb, and one unknown limb); and one MC fracture (left limb). DNA samples from 24 healthy and injury-free racing Greyhounds (12 males and 12 females) were selected as controls.

In order to reduce bias and to limit the effect of population stratification on results, individuals sampled for GWAS should be selected from the same

underlying population (Anderson *et al.*, 2010). In the present study, case and control individuals were matched by breed and were sampled from the same population. All Greyhounds included in the study were associated with one GBGB-licensed Greyhound stadium in the North-West of the UK. Due to the limited availability of samples, it was not possible to perform further matching of case and control individuals.

A case-control study design for GWAS is based on the assumption that the individuals in the study population are 'unrelated', in that the maximum familial relationship between any two individuals is less than that of a second-degree relative (Anderson *et al.*, 2010). Following DNA extraction and quantification for all biological samples collected in the study, selection of DNA samples to be included in the GWAS was limited (by DNA concentration) to those obtained from Greyhound saliva samples. The six-generation pedigree of each Greyhound with a salivary DNA sample was examined, and DNA samples were selected based on suitability of the dog for meeting or exceeding the specified case or control criteria, the quality and quantity of the DNA sample, and at the same time ensuring that all Greyhounds selected for the GWAS were as unrelated as possible. Phenotypic data for the case and control individuals are summarised in Appendix 9.

The selected DNA samples were thawed and vortexed briefly, and 10 µl of each sample transferred to a PCR tray as displayed in Table 5.1. Case and control

samples were allocated to alternate wells in the plate rather than two separate blocks, to reduce the risk of bias in the genotyping procedure.

	1	2	3	4	5	6	
A	S044	S106	S184	S097	S026	S006	Case
B	S036	S111	S116	S193	S177	S147	Control
C	S047	S107	S185	S029	S073	S079	Case
D	S058	S123	S180	S178	S105	S054	Control
E	S122	S125	S150	S081	S095	S088	Case
F	S066	S148	S143	S060	S082	S086	Control
G	S085	S171	S053	S025	S108	S001	Case
H	S076	S146	S033	S043	S074	S109	Control

Key:

Female
Male

Table 5.1. The allocation of racing Greyhound DNA samples to 48 wells in a PCR plate for the Genome-Wide Association Study (GWAS). Case (affected) and control (unaffected) samples were allocated to alternate wells, with male (blue) and female (pink) samples mixed on an ad hoc basis.

5.3.4. Illumina® Genotyping

Genome-wide SNP genotyping of the 48 Greyhound DNA samples was performed using the Illumina® CanineHD BeadChip and Infinium HD Ultra Assay (Illumina, San Diego, California, USA), with genotyping and imaging carried out at the Arthritis Research UK Centre for Genetics and Genomics at the University of Manchester (Manchester, UK).

The second generation Illumina® CanineHD BeadChip provides genome-wide coverage using 173,662 evenly spaced and validated SNPs derived from the

CanFam2.0 canine reference sequence assembly, with an average genome coverage of greater than 70 SNPs per megabase (Mb). The CanineHD BeadChip was developed by Illumina in collaboration with the LUPA Consortium (Lequarre *et al.*, 2011), which includes 22 European universities and other partners. SNPs were selected from the 2.5 million SNP set created by the Dog Genome Project, and a hybridisation-based targeted re-sequencing method of SNP discovery was used within gaps in the SNP map, to identify a further 1,696 SNPs. The Illumina® CanineHD BeadChip provides greater than 99% average call rates and a high accuracy of 99.99% reproducibility. The SNP content of the Illumina® CanineHD BeadChip is highly polymorphic, with SNPs selected from a diverse set of dog breeds, therefore enabling the investigation of genetic variation and disease mapping in all domestic breeds of dog.

Genotyping was performed according to the manufacturer's protocol (Illumina, Inc., 2009). Briefly, the DNA sample was denatured and neutralised prior to overnight isothermal whole-genome amplification. The amplified product was then fragmented using a controlled enzymatic procedure, to avoid over-fragmentation of the sample. The fragmented DNA was precipitated using 100% isopropanol, then collected by centrifugation at 4°C. Following re-suspension of the precipitated DNA in a hybridisation buffer, the DNA was annealed to locus-specific probes on the Illumina® CanineHD BeadChip and incubated at 48°C for 16 hours. The BeadChip was then washed to remove unhybridised and non-specifically hybridised DNA. Single-base extension of the oligos attached to the BeadChip was performed, using the captured DNA as a template, to incorporate

detectable labels on the BeadChip and determine the genotype call for the sample. Using a technique based on immunohistochemistry, the BeadChips were stained in order to increase the intensity of the signal. The BeadChips were washed and coated, then imaged and read using the Illumina® iScan System (Illumina, San Diego, California, USA) array scanner, which records high-resolution images of the light emitted from the fluorophores of the single-base extension product on the BeadChip. Finally, the fluorescent intensity data were output to Illumina GenomeStudio™ software, and automated SNP calling was performed using the GenCall software algorithm.

Genotyping data was analysed using the freely available, open-source whole genome association analysis software PLINK, version 1.07 (Purcell *et al.*, 2007; Purcell, 2009). Graphical representation of the data was performed using the software packages Haploview, version 4.2 (Barrett *et al.*, 2005) and R, version 3.1.1 (The R Foundation for Statistical Computing, 2014).

5.3.5. Data Cleaning and Quality Control

The raw genotypic data were input to the whole-genome association analysis software PLINK, version 1.07 (Purcell *et al.*, 2007; Purcell, 2009). Prior to statistically testing for association, the data were assessed and filtered in order to identify and exclude DNA samples and SNPs that may introduce bias. The X-chromosome heterozygosity rate was used to check that the estimated gender of an individual, based on genotyping data, was concordant with their reported

gender. The sample call rate is the proportion of called SNPs per sample out of the total number of SNPs in the data set. Individuals with a sample call rate < 92% were excluded, resulting in the removal of three case (affected) individuals due to low genotyping. The genotype call rate refers to the proportion of genotypes per SNP with non-missing data, and the minor allele frequency is the frequency of the least common allele at a given locus in a given population. SNPs with a genotyping call rate < 95% and/or a minor allele frequency < 0.01 were excluded, resulting in exclusion of 19,909 SNPs and 53,951 SNPs, respectively.

The Hardy-Weinberg equilibrium states that gene and genotype frequencies will remain constant from one generation to the next in a large random-mating, homogenous population with no selection, mutation or migration (Falconer and Mackay, 1996; Simm, 1998). If the gene frequencies of two alleles at a biallelic locus in Hardy-Weinberg equilibrium are p and q , and given a minor allele frequency of q , the frequencies of the three possible genotypes aa , Aa and AA , are $(1 - q)^2$, $2q(1 - q)$ and q^2 , respectively (Anderson *et al.*, 2010; Falconer and Mackay, 1996). A total of 75 SNPs failed the Hardy-Weinberg Equilibrium test ($p \leq 0.0001$) in the control population, and were therefore excluded.

After filtering, frequency and genotyping quality control and assessment for population stratification, 21 cases and 24 controls (23 males and 22 females) and a total of 105,431 SNPs remained, to be included in further analysis.

5.3.6. Genome-Wide Association Analysis

The data were analysed for genome-wide association using a case-control allelic test in PLINK, version 1.07 (Purcell *et al.*, 2007; Purcell, 2009), using the commands `--assoc` and `--dog`, based on comparing allele frequencies between Greyhounds with (cases) and without (controls) a stress fracture. The command `--adjust` was used to apply the Bonferroni correction for multiple testing.

The position of associated SNPs on the chromosome and potential causative genes were identified from the CanFam2.0 and CanFam3.1 canine genome assembly using the Ensembl online public database (Ensembl, 2014). Graphical representation of the data was performed using the software packages Haploview, version 4.2 (Barrett *et al.*, 2005) and R, version 3.1.1 (The R Foundation for Statistical Computing, 2014).

5.4. Results

5.4.1. Creation of a DNA archive of British Racing Greyhounds

A total of 237 DNA samples (135 males and 102 females) were obtained, extracted, quantified and stored to create a racing Greyhound DNA archive for use in the present study and for future genetic research. 91 of the DNA samples (55 males and 36 females) were obtained from Greyhounds that had sustained a stress fracture of the tarsus ($n = 83$ dogs), metatarsus ($n = \text{six}$ dogs) or metacarpus ($n = \text{two}$ dogs), and 146 of the samples (80 males and 66 females) were obtained from healthy racing Greyhounds that had never sustained an orthopaedic injury. Detailed phenotypic, performance, pedigree and injury data were obtained for each DNA sample within the archive.

5.4.2. Genome-Wide Association Study

Following analysis for genome-wide association in PLINK, a quantile-quantile (Q-Q) plot of the data was produced (Figure 5.1) using the statistical software R, version 3.1.1 (The R Foundation for Statistical Computing, 2014), to compare the observed distribution of the GWA test statistics with the expected null distribution. The Q-Q plot is a graphical representation of $-\log_{10} p$ -values from a logistic regression for all 105,431 SNPs that passed the quality control criteria, under a multiplicative model of association. The Q-Q plot (Figure 5.1) illustrates minor deviations in observed results from the null distribution, except in the upper tail of the distribution which

correspond to the most statistically significant SNPs (Clarke *et al.*, 2011). As the majority of results are shown to follow the null distribution, this suggests that population stratification is unlikely to be of concern in the present study (Clarke *et al.*, 2011). The genomic inflation factor (λ) based on median chi-squared was 1.45.

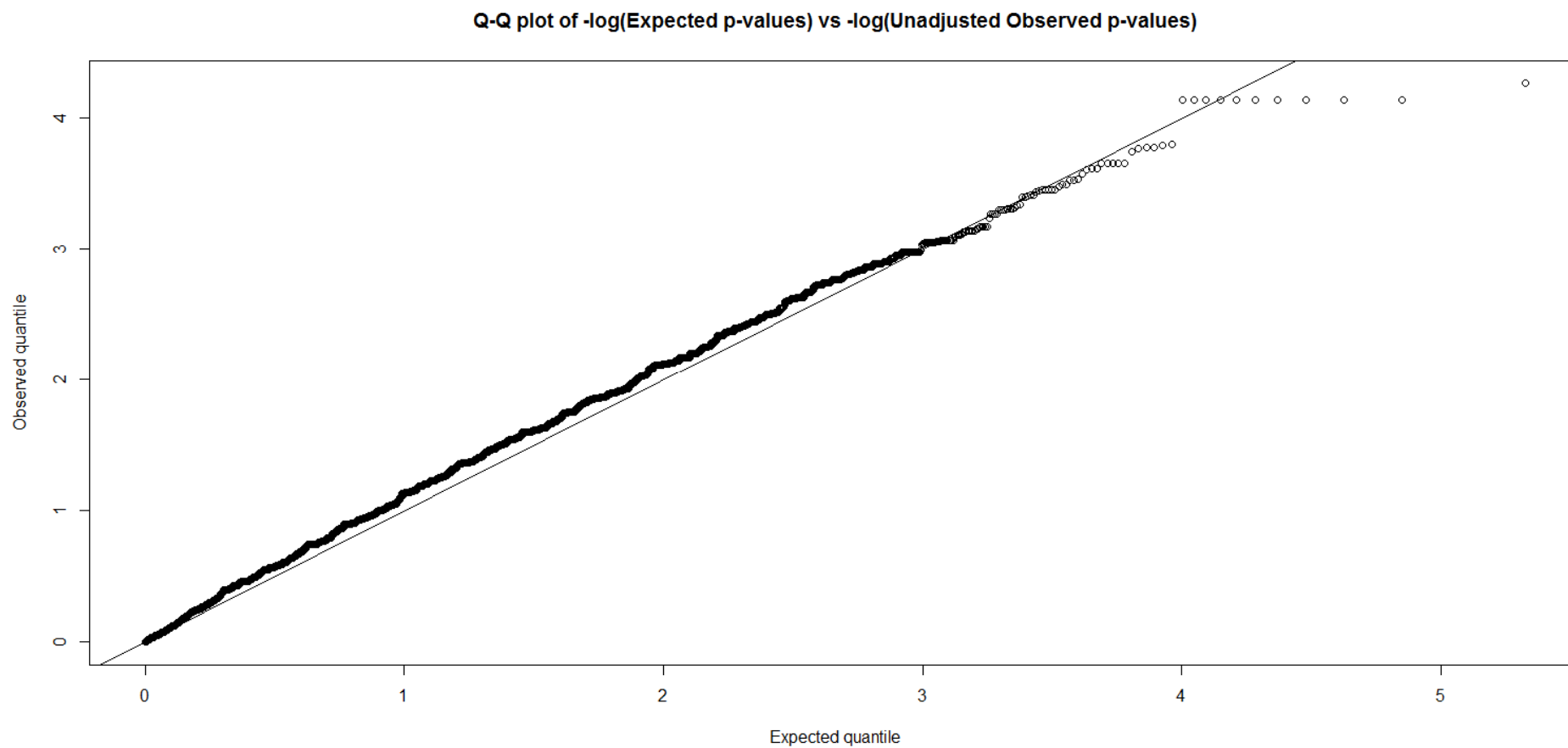


Figure 5.1. A quantile-quantile (Q-Q) plot of $-\log(\text{expected } p\text{-values})$ and $-\log(\text{Unadjusted observed } p\text{-values})$ for the Genome-Wide Association Study (GWAS) of stress fracture injuries in the racing Greyhound.

To determine whether SNPs are significant, human GWA studies use a significance threshold in which a p -value ≤ 0.0000007 is considered as very highly associated and a p -value ≤ 0.00001 considered as reasonably associated with the phenotype. A significance threshold set at $p \leq 0.00001$ has been considered suitable to assess the association of SNPs in canine GWA studies (Baird *et al.*, 2014; Wood *et al.*, 2009), due to the extended linkage disequilibrium in the canine genome. Any association of SNPs in which $p \leq 0.00001$ would be considered as associated with disease susceptibility, and would be confirmed by fine-mapping in a validation study. Previous canine GWA studies have, however, identified and selected SNPs with lower significance thresholds of $p \leq 0.0001$ or $p \leq 0.001$ for further genotyping in a larger cohort, and subsequently found significant associations (Massey *et al.*, 2014).

Using a significance threshold set at $p \leq 0.00001$, any association of SNPs in the present study in which $p \leq 0.00001$ would be considered as associated with disease susceptibility and therefore an increased risk of stress fracture. Following analysis of the GWAS data, no SNPs were found to reach genome-wide statistical significance at the $p \leq 0.00001$ significance level. 11 SNPs were significantly associated at the $p \leq 0.0001$ level, however, and a further 96 SNPs were significantly associated at the $p \leq 0.001$ level. No SNPs were statistically significant following Bonferroni corrections to adjust for multiple testing.

A whole-genome association plot of significance (Manhattan plot) was produced using Haploview, version 4.2 (Barrett *et al.*, 2005), to display the association test

$-\log_{10}$ unadjusted p -values for each SNP (CanFam3.1) as a function of chromosomal location (Figure 5.2). Each chromosome is represented on the plot by a different colour. The SNPs most significantly associated with stress fracture in racing Greyhounds correspond to those with the greatest $-\log_{10} p$ -values (Clarke *et al.*, 2011). The blue and red horizontal lines indicate a significance threshold level of $p \leq 0.001$ and $p \leq 0.0001$, respectively. The Manhattan plot illustrates that SNPs present on chromosomes 20, 22 and 27 are associated with stress fracture in racing Greyhounds at the $p \leq 0.0001$ significance level, based on the CanFam3.1 canine genome assembly. An additional number of SNPs present on various chromosomes are associated with stress fracture at the $p \leq 0.001$ significance level.

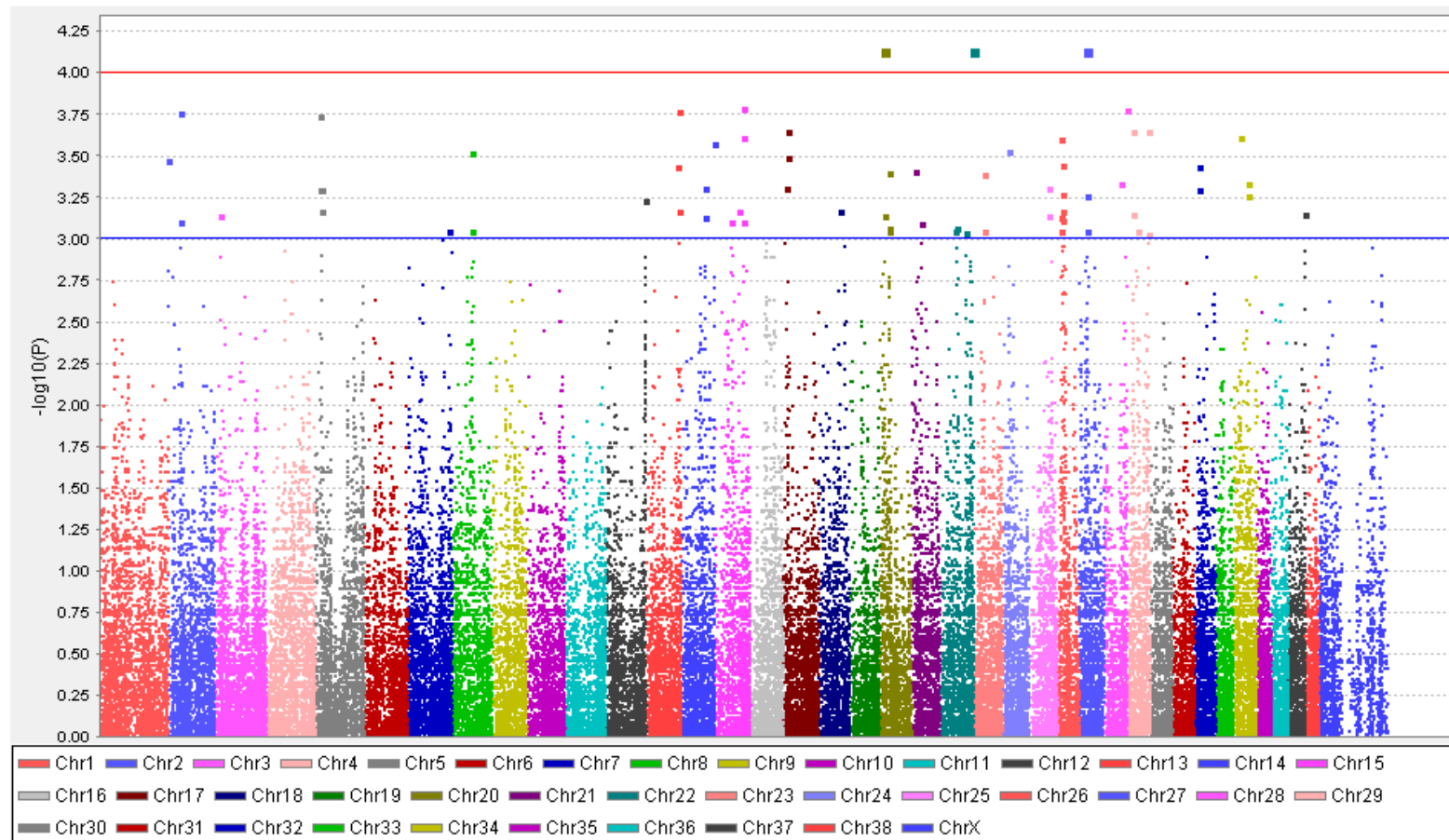


Figure 5.2. A Manhattan Plot for racing Greyhounds following genome-wide association analysis. The blue and red horizontal lines indicate a significance threshold level of $p \leq 0.001$ and $p \leq 0.0001$, respectively, for SNPs (CanFam3.1) associated with stress fracture in racing Greyhounds. Each SNP is represented by its $-\log_{10} p$ -value and chromosomal location. Key: Chr = Chromosome.

The GWAS analysis identified chromosome positions of SNPs based on the CanFam3.1 canine genome assembly. The most significantly associated SNP (BICF2P1266953), as well as the 12th ranking SNP (BICF2P252391), were initially identified as unmapped regions with an unknown chromosome (0) and position (0). Consequently, the $-\log_{10} p$ -values for these two SNPs are missing from the Manhattan plot graphical representation of the GWAS results (Figure 5.2). A search of the previous CanFam2.0 version of the canine genome assembly, investigating whether the SNP chromosomal positions had been identified on the preceding SNP map, subsequently found both SNPs to be located on the X-chromosome.

SNPs associated with stress fracture in racing Greyhounds at the $p \leq 0.0001$ significance level were present on the X-chromosome (1 SNP) (Figure 5.3), and chromosomes 20 (6 SNPs) (Figure 5.4 and Figure 5.5), 22 (2 SNPs) and 27 (2 SNPs) (Figure 5.6). Additional associated SNPs at the $p \leq 0.001$ significance level were present on the X-chromosome (1 SNP), and chromosomes 2 (3 SNPs), 3 (4 SNPs), 5 (5 SNPs), 7 (1 SNP), 8 (7 SNPs), 12 (1 SNP), 13 (3 SNPs), 14 (3 SNPs), 15 (6 SNPs), 17 (7 SNPs), 18 (1 SNP), 20 (13 SNPs), 21 (3 SNPs), 22 (4 SNPs), 23 (3 SNPs), 24 (1 SNP), 25 (2 SNPs), 26 (11 SNPs), 27 (2 SNPs), 28 (2 SNPs), 29 (6 SNPs), 32 (2 SNPs), 34 (4 SNPs) and 37 (1 SNP).

The position of associated SNPs on the chromosome and potential causative genes were identified from the CanFam2.0 and CanFam3.1 canine genome assembly using the Ensembl online public database (Ensembl, 2014).

The allelic odds ratio (OR) describes the association between disease and allele by comparing the odds of disease (stress fracture) in an individual carrying the minor (second most common) allele $A1$ to the odds of disease in an individual carrying the major (most common) allele $A2$ (Clarke *et al.*, 2011). The data for each SNP with minor allele $A1$ and major allele $A2$ in case and control groups containing n individuals can be represented as a 2 x 2 contingency table of disease status by allele count (Table 5.2).

Allele	$A1$	$A2$	Total
Cases	m_{11}	m_{12}	$m_{1\cdot}$
Controls	m_{21}	m_{22}	$m_{2\cdot}$
Total	$m_{\cdot 1}$	$m_{\cdot 2}$	$2n$

Table 5.2. The genome-wide association study (GWAS) data for each single nucleotide polymorphism (SNP) with minor (second most common) allele $A1$ and major (most common) allele $A2$ in case and control groups containing n individuals can be represented as a 2 x 2 contingency table of disease status by allele count.

The allelic OR for the minor allele $A1$ is estimated by:

$$OR_{A1} = (m_{11}m_{22}) / (m_{12}m_{21})$$

(Clarke *et al.*, 2011)

Under the null hypothesis of no genetic association of allele with the disease (stress fracture), the relative allele frequencies are expected to be equal in both case and control groups. An allelic association test is therefore based on a simple chi-squared test for independence of rows and columns of the contingency table (Table 5.2):

$$\chi^2 = \sum_{i=1}^2 \sum_{j=1}^2 \frac{(m_{ij} - E[m_{ij}])^2}{E[m_{ij}]} \quad \text{Where: } E[m_{ij}] = \frac{m_{i.} m_{.j}}{2n}$$

χ^2 has a chi-squared distribution with one degree of freedom under the null hypothesis of no association (Clarke *et al.*, 2011).

The top 50 most significantly associated SNPs ($p \leq 0.001$) identified in the present study GWAS analysis are displayed in Table 5.3. Details are provided of the chromosome and position (base pair) of each identified SNP on the canine genome assembly SNP map (CanFam3.1 or CanFam2.0), frequency of the minor allele *A1* in cases and controls, basic allelic test chi-square value (one degree of freedom), asymptotic *p*-value for this test and the estimated odds ratio for the minor allele *A1*.

Table 5.3. Racing Greyhound stress fracture GWAS results: the top 50 most significantly associated SNPs ($p \leq 0.001$) from the GWAS analysis. **Key:** Rank = order of statistical significance [1 (lowest p -value) to 50 (highest p -value)]; SNP = Identity of SNP marker; Chr = Chromosome; Position = Physical location (base-pair) of the SNP based on the CanFam3.1 or CanFam2.0 canine genome assembly SNP map; A1 = Minor (second most common) allele name (based on whole sample); F_A = Frequency of minor allele in cases; F_U = Frequency of minor allele in controls; A2 = major (most common) allele name; CHISQ = Basic allelic test chi-square (1 degree of freedom); p -value = Asymptotic p -value for this test; OR = Estimated odds ratio for A1 (minor allele).

Rank	SNP	Chr	Position (bp) CanFam3.1 (unless stated otherwise)	A1	F_A	F_U	A2	CHISQ	p -value	OR
1	BICF2P1266953	X	100823429 (CanFam2.0)	G	0.6905	0.2609	A	16.28	0.0000545	6.321
2	BICF2P1136919	20	6030423	C	0.6667	0.25	T	15.75	0.0000723	6
3	BICF2S22910736	20	6046176	T	0.6667	0.25	C	15.75	0.0000723	6
4	BICF2P303083	20	6051952	G	0.6667	0.25	T	15.75	0.0000723	6
5	BICF2P1456588	20	6067545	T	0.6667	0.25	C	15.75	0.0000723	6
6	BICF2P1384557	20	6077152	T	0.6667	0.25	C	15.75	0.0000723	6
7	BICF2P1242669	20	6080783	A	0.6667	0.25	G	15.75	0.0000723	6
8	BICF2S23433818	27	11906685	G	0.6667	0.25	A	15.75	0.0000723	6
9	BICF2G630142608	27	12568738	A	0.6667	0.25	G	15.75	0.0000723	6
10	BICF2P229977	22	57627954	A	0.619	0.2083	G	15.74	0.0000725	6.175
11	BICF2G63089383	22	57646359	G	0.619	0.2083	T	15.74	0.0000725	6.175

Table 5.3. Continued:

Rank	SNP	Chr	Position (bp) CanFam3.1 (unless stated otherwise)	A1	F_A	F_U	A2	CHISQ	p-value	OR
12	BICF2P252391	X	100811736 (CanFam2.0)	C	0.6905	0.2917	T	14.28	0.0001576	5.418
13	BICF2P647060	15	51461868	T	0.3095	0.02083	C	14.21	0.0001633	21.07
14	BICF2S23110295	28	39987716	G	0.1429	0.5208	A	14.17	0.0001668	0.1533
15	TIGRP2P184870_rs9068575	13	60474403	C	0.6667	0.2708	T	14.15	0.0001687	5.385
16	BICF2S23530035	2	22814727	C	0.5952	0.2083	T	14.11	0.0001725	5.588
17	BICF2P1024144	5	10561319	T	0.02381	0.3333	A	14.01	0.0001821	0.04878
18	BICF2P381996	17	7630555	A	0.381	0.0625	G	13.64	0.0002214	9.231
19	BICF2G630222626	17	7791434	G	0.381	0.0625	A	13.64	0.0002214	9.231
20	BICF2G630222618	17	7796930	C	0.381	0.0625	T	13.64	0.0002214	9.231
21	TIGRP2P364726_rs8713314	29	11167861	T	0.381	0.0625	C	13.64	0.0002214	9.231
22	BICF2P313893	29	39107083	G	0.381	0.0625	A	13.64	0.0002214	9.231
23	BICF2P658495	15	50425208	C	0.4762	0.125	T	13.45	0.0002452	6.364
24	BICF2P389418	34	13037291	T	0.4762	0.125	A	13.45	0.0002452	6.364
25	BICF2P784071	26	7664505	C	0.5476	0.1739	T	13.44	0.0002467	5.75
26	BICF2P354264	14	60637439	T	0	0.2708	C	13.3	0.0002661	0
27	BICF2G630509638	24	11506995	T	0.5	0.1458	C	13.11	0.0002937	5.857

Table 5.3. Continued:

Rank	SNP	Chr	Position (bp) CanFam3.1 (unless stated otherwise)	A1	F_A	F_U	A2	CHISQ	p-value	OR
28	BICF2P1066080	8	37193959	C	0.09524	0.4375	A	13.08	0.0002985	0.1353
29	BICF2P1043265	8	37258896	G	0.09524	0.4375	C	13.08	0.0002985	0.1353
30	BICF2P108099	17	8787864	G	0.4048	0.08333	A	12.94	0.0003222	7.48
31	BICF2G630222625	17	7792124	G	0.381	0.06522	A	12.93	0.0003237	8.821
32	BICF2P1199117	2	896834	T	0.2381	0	G	12.86	0.0003362	NA
33	BICF2G630806983	26	9230813	A	0.07143	0.3958	C	12.76	0.0003534	0.1174
34	BICF2G630806969	26	9244378	T	0.07143	0.3958	C	12.75	0.0003534	0.1174
35	BICF2G630806927	26	9274920	A	0.07143	0.3958	G	12.75	0.0003534	0.1174
36	BICF2G630806811	26	9369339	T	0.07143	0.3958	C	12.76	0.0003534	0.1174
37	BICF2G630806803	26	9381354	T	0.07143	0.3958	C	12.76	0.0003534	0.1174
38	BICF2P264854	13	56621167	A	0.2857	0.02083	T	12.72	0.0003623	18.8
39	TIGRP2P378557_rs8795056	32	9375097	T	0	0.2609	C	12.69	0.0003683	0
40	BICF2P117167	21	7884602	A	0.2143	0.5833	G	12.6	0.0003854	0.1948
41	BICF2G630658037	21	7901756	C	0.2143	0.5833	T	12.6	0.0003854	0.1948
42	BICF2S22912150	20	17619521	G	0.5714	0.2083	A	12.56	0.0003934	5.067
43	BICF2G630382857	23	18299381	A	0.5952	0.2292	G	12.51	0.0004053	4.947

Table 5.3. Continued:

Rank	SNP	Chr	Position (bp) CanFam3.1 (unless stated otherwise)	A1	F_A	F_U	A2	CHISQ	<i>p</i> -value	OR
44	BICF2G630382851	23	18301721	T	0.5952	0.2292	G	12.51	0.0004053	4.947
45	BICF2P1190529	28	29176458	C	0.119	0.4583	T	12.28	0.0004581	0.1597
46	BICF2G630459529	34	25748568	T	0.4524	0.1136	C	12.26	0.0004638	6.443
47	TIGRP2P330196_rs8996086	25	37057746	A	0.04762	0.3478	G	12.16	0.0004881	0.09375
48	BICF2P90099	14	42981679	G	0.3571	0.0625	A	12.15	0.0004898	8.333
49	BICF2S23754865	17	4680896	G	0.3571	0.0625	A	12.15	0.0004898	8.333
50	BICF2S23754864	17	4681082	G	0.3571	0.0625	C	12.15	0.0004898	8.333

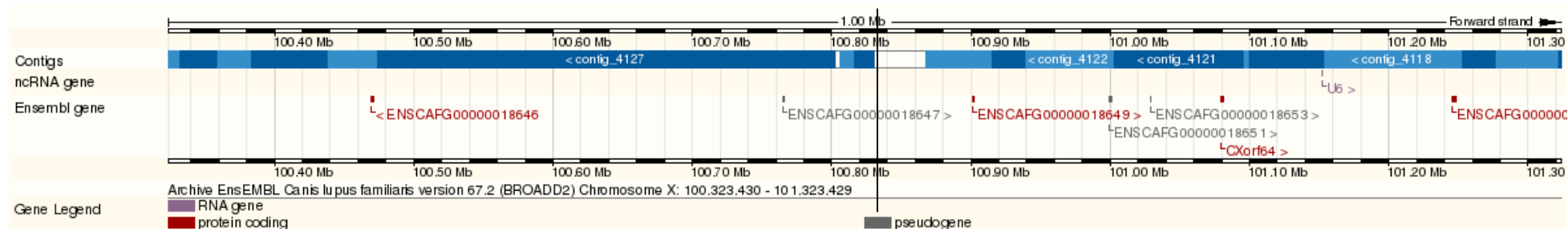


Figure 5.3. Ensembl (CanFam2.0) chromosome view of SNP BICF2P1266953 at base pair position 100823429 on chromosome X.

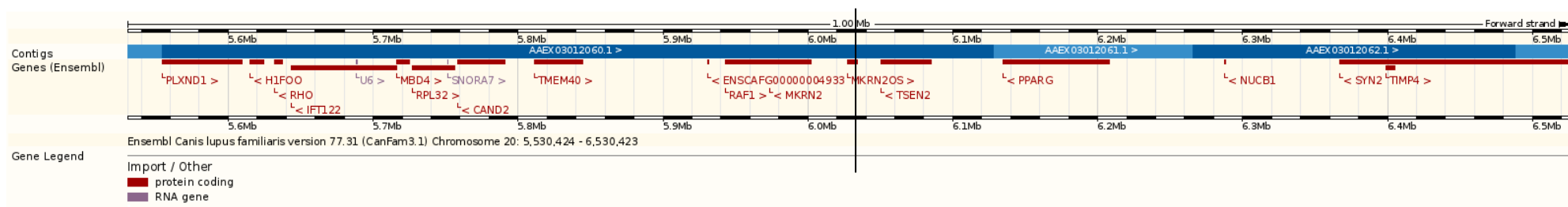


Figure 5.4. Ensembl (CanFam3.1) chromosome view of SNP BICF2P1136919 at base pair position 6030423 on chromosome 20.

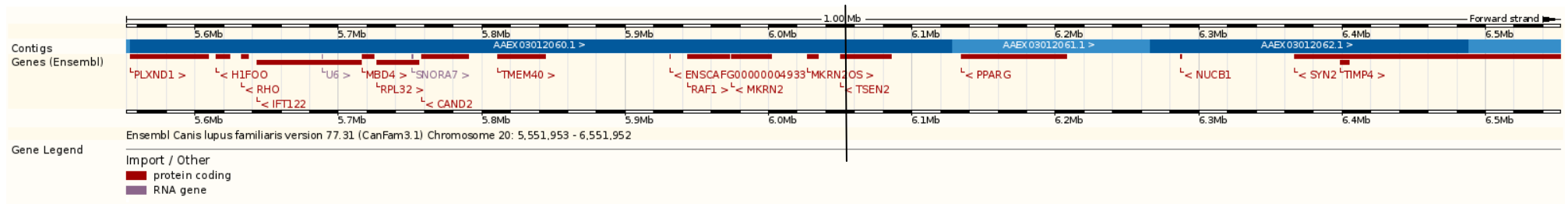


Figure 5.5. Ensembl (CanFam3.1) chromosome view of SNP BICF2P303083 at base pair position 6051952 on chromosome 20.

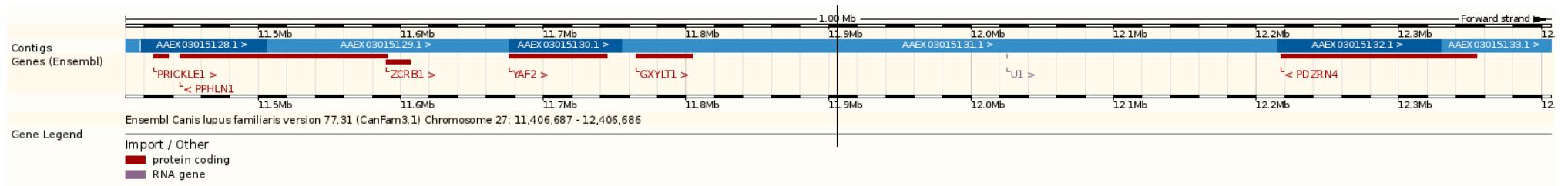


Figure 5.6. Ensembl (CanFam3.1) chromosome view of SNP BICF2S23433818 at base pair position 11906685 on chromosome 27.

5.5. Discussion

A total of 237 Greyhound DNA samples (135 males and 102 females) from Greyhounds that had sustained a stress fracture (n = 91 dogs) and from healthy uninjured dogs (n = 146 dogs) were obtained, extracted, quantified and stored at -40°C, to create the first British racing Greyhound DNA archive. Individual Greyhounds were well phenotyped, with detailed pedigree, phenotypic, race history and injury data recorded for each dog, therefore providing a valuable resource for future scientific research. The present study is the first to investigate genetic factors that may predispose racing Greyhounds to developing stress fracture injuries, by performing a case-control GWAS to identify potentially associated SNPs.

5.5.1. Greyhound DNA Samples

91 of the total DNA samples (55 males and 36 females) were obtained from Greyhounds that sustained a stress fracture of the tarsus (n = 83), metatarsus (n = 6) or metacarpus (n = 2). Of the 83 tarsal fractures, 74.70% (n = 62) involved the right pelvic limb, 8.43% (n = 7) involved the left pelvic limb, 2.41% (n = 2) involved both right and left pelvic limbs, and 14.46% (n = 12) involved an unknown limb. These results are consistent with previous reports of tarsal fractures in racing Greyhounds, in which the vast majority (up to 96%) occurred in the right pelvic limb (Boudrieau *et al.*, 1984a; Davis, 1967; Devas, 1961; Gannon, 1972; Guilliard, 2000; Keene and Yarborough, 1966; Prole, 1976). This is likely to be due to the

stresses placed on the right tarsal joint during anticlockwise racing at high speeds (Anderson *et al.*, 1995).

5.5.2. Sample Collection and DNA Extraction

A quantity of 4 µl of DNA at a minimum concentration of 20 ng/µl per sample was required for Illumina® genome-wide SNP genotyping. None of the DNA samples extracted from Greyhound muscle or skin tissue contained a sufficient concentration of DNA to be suitable for inclusion in the GWA study. The DNA obtained, however, would still be suitable for validation of the GWAS results by fine-mapping, and for use in other types of genetic studies.

All muscle and skin tissue samples were collected post-mortem from Greyhounds euthanased at the track due to injury. Time restrictions during the race meeting prevented the immediate collection of muscle samples by the track veterinary surgeon. Consequently, the low yield of DNA obtained from the muscle tissue samples may have been due to the necessity to freeze the Greyhound immediately after euthanasia at the track, with muscle sample collection taking place at a later date following thawing of the cadaver. The muscle tissue samples were therefore subjected to at least two freeze-thaw cycles prior to DNA extraction, which may have resulted in some DNA degradation.

For comparison, fresh muscle tissue samples were collected from two non-Greyhound breed dogs, euthanased for reasons unrelated to the study. DNA

extraction was performed using the Qiagen DNeasy Blood and Tissue kits and following the same protocol detailed in Chapter Two. The samples resulted in considerably higher concentrations of DNA compared to those obtained for the Greyhounds. This suggests that freezing of the Greyhound tissue and subsequent freeze-thawing for sample collection and DNA extraction is likely to have resulted in some DNA degradation.

Due to the difficulties of obtaining a sufficient quantity of DNA for genome-wide SNP genotyping from the muscle samples, an alternative method of DNA collection was trialled. The track veterinary surgeon was provided with skin punch biopsy kits to enable collection of Greyhound tissue samples quickly and easily during the immediate post-mortem period, before freezing of the cadaver (Chapter 2.2.1.3). Overall, skin biopsy tissue samples were submitted for only two Greyhounds. Each sample had been stored in 500 µl DNaGard Tissue solution (Biomatrica, USA) to preserve the tissue and DNA until further processing of the sample. None of the skin tissue samples, however, contained a sufficient quantity of DNA for genome-wide SNP genotyping. Interestingly, a considerably lower DNA yield was obtained from the Greyhound skin biopsy samples compared to DNA obtained from a similar quantity of fresh canine (non-Greyhound) skin tissue collected and extracted immediately post-mortem. This suggests that the DNaGard Tissue Solution (Biomatrica, USA) may not be suitable for the preservation of tissue and DNA samples for the purposes of inclusion in GWA studies, in which a relatively high quantity and quality of DNA per sample is required.

All Greyhound saliva samples were collected by the author using Oragene®-Animal OA-400 saliva collection kits (Oragene®, DNA Genotek Inc., Ontario, Canada) to ensure consistency in the procedure. There was considerable variation in the quantity of DNA obtained per sample (1.23 ng/ml to 118 ng/ml). In contrast to the muscle and skin tissue samples, however, the majority of saliva samples collected contained a sufficient quantity and quality of DNA to be included in the GWA study. The Oragene®-Animal saliva collection kits were therefore found to be an easy and effective method of obtaining canine DNA samples suitable for use in GWA studies. All of the case (affected) and control (unaffected) DNA samples selected for Illumina® genotyping in the present study were samples extracted from Greyhound saliva collected using the Oragene® Animal kits.

5.5.3. Single Nucleotide Polymorphisms (SNPs) and Chromosomal Regions of Interest

The present study represents the first genetic investigation of stress fracture injuries in the racing Greyhound. GWA studies use an unbiased, hypothesis-free, whole genome approach for identification of genetic variations associated with a complex disease such as stress fracture. Consequently, a GWAS methodology was selected rather than a candidate gene association study approach, as there were no pre-determined specific genes or loci of interest linked to Greyhound stress fracture by previous research.

Using a small number of samples (21 cases and 24 controls), the discovery GWAS identified a number of SNPs that were nominally associated with stress fracture in

the racing Greyhound, and that warrant further investigation. 11 SNPs were found to be associated with stress fracture at the $p \leq 0.0001$ significance level. An additional 96 SNPs were associated at the $p \leq 0.001$ significance level. The identified SNPs, regions of chromosomes and potentially associated genes are summarised in Table 5.4 for all associated SNPs at the $p \leq 0.0001$ significance level. An additional nine associated SNPs of interest at the $p \leq 0.001$ significance level are summarised in Table 5.5. Further work would be required to validate the results of the GWAS, by sequencing of these identified regions to confirm associations.

Rank	SNP	Chr	Location
1	BICF2P1266953	X	Intergenic region between two uncharacterised protein-coding genes
2	BICF2P1136919	20	Intragenic region within the uncharacterised protein-coding gene C3orf83 (chromosome 3 open reading frame 83)
3	BICF2S22910736	20	Intergenic region between C3orf83 and TSEN2 (tRNA-splicing endonuclease subunit)
4	BICF2P303083	20	Intragenic region within the TSEN2 gene, and just upstream of the protein-coding gene PPARG (peroxisome proliferator-activated receptor gamma)
5	BICF2P1456588		
6	BICF2P1384557		
7	BICF2P1242669		
8	BICF2S23433818	27	Intergenic region between GXYLT1 (glucoside xylosyltransferase 1) and PDZRN4 (PDZ domain containing ring finger 4)
9	BICF2G630142608	27	Intergenic region between PDZRN4 and CNTN1 (contactin 1)
10	BICF2P229977	22	Intragenic region within the MYO16 (Myosin XVI) gene
11	BICF2G63089383		

Table 5.4. Racing Greyhound stress fracture GWAS results: Single Nucleotide Polymorphisms (SNPs) associated with stress fracture at the $p \leq 0.0001$ significance level and their location on the chromosome. Where: Rank = overall order of statistical significance [1 (lowest p -value) to 11 (highest p -value)]; Chr = Chromosome; Location = physical location of the SNP on the chromosome, based on the CanFam3.1 or CanFam2.0 genome assembly.

Rank	SNP	Chr	Location
12	BICF2P252391	X	Intergenic region between two uncharacterised protein-coding genes ENSCAFG00000018646 and ENSCAF00000018649
13	BICF2P647060	15	Intragenic region within the RNF175 (ring finger protein 175) gene, and just upstream of the protein-coding gene SFRP2 (Canis lupus familiaris secreted frizzled-related protein 2)
14	BICF2S23110295	28	Intergenic region between TCERG1L (transcription elongation regulator 1-like) and PPP2R2D (protein phosphatase 2, regulatory subunit B, delta)
15	TIGRP2P184870_rs9068575	13	Intragenic region within the SLC4A4 [solute carrier family 4 (sodium bicarbonate cotransporter), member 4] gene
16	BICF2S23530035	2	Intragenic region within the FRMD4A (FERM domain containing 4A) gene
17	BICF2P1024144	5	Intergenic region just downstream of the OR4D5 (Olfactory receptor, family 4, subfamily D, member 5) gene
18	BICF2P381996	17	Intragenic region within the ATP6V1C2 (ATPase, H ⁺ transporting, lysosomal 42kDa, V1 subunit C2) gene
19	BICF2G630222626	17	Intergenic region, just downstream of the KCNF1 (potassium voltage-gated channel, subfamily F, member 1) gene
20	BICF2G630222618		

Table 5.5. Racing Greyhound stress fracture GWAS results: Single Nucleotide Polymorphisms (SNPs) of interest associated with stress fracture at the $p \leq 0.001$ significance level and their location on the chromosome. Where: Rank = overall order of statistical significance [12 (lowest p -value) to 20 (highest p -value)]; Chr = Chromosome; Location = physical location of the SNP on the chromosome, based on the CanFam3.1 or CanFam2.0 genome assembly.

At the $p \leq 0.0001$ significance level, the most statistically significant SNP (lowest p -value) was BICF2P1266953 located on the X-chromosome (Figure 5.3), in an intergenic region between two uncharacterised protein-coding genes (ENSCAFG00000018646 and ENSCAFG00000018649) and upstream of the uncharacterised protein-coding gene CXorf64 (chromosome-X open reading frame 64).

The second most statistically significant SNP was BICF2P1136919, located on chromosome 20 (Figure 5.4) within the uncharacterised protein-coding gene MKRN2OS, otherwise known as C3orf83 (chromosome 3 open reading frame 83). The third most significant SNP was BICF2S22910736, located on chromosome 20 in an intergenic region between C3orf83 and TSEN2 (tRNA-splicing endonuclease subunit), which catalyses the removal of introns in the first step in RNA splicing. The fourth, fifth, sixth and seventh most significant SNPs were BICF2P303083 (Figure 5.5), BICF2P1456588, BICF2P1384557 and BICF2P1242669, respectively, each located on chromosome 20 within the TSEN2 gene. The second to seventh most significant SNPs were all located on chromosome 20 with the same minor allele frequency of 0.6667 in case (affected) samples, and are therefore likely to be in linkage disequilibrium. Each of these SNPs were located just upstream of the protein-coding gene PPARG (peroxisome proliferator-activated receptor gamma); a type II nuclear receptor protein that functions as a ligand-activated transcription factor regulating the expression of genes. PPARG heterodimerises with the retinoid X receptor (RXR) and binds to specific regions on the DNA of target genes, and is thought to regulate adipocyte differentiation and glucose homeostasis

(Tontonoz and Spiegelman, 2008). An important paralog of the PPARG gene is the Vitamin D Receptor (VDR) gene, and RXR is a common heterodimeric binding partner of both nuclear receptors. Polymorphisms in the VDR gene have been implicated in the pathogenesis of stress fractures in human military recruits (Chatzipapas *et al.* 2009).

PPARG has been identified as a key modulator of skeletal remodelling and bone homeostasis. Stimulation of bone marrow mesenchymal stem cells by PPARG causes differentiation of the stem cells into adipocytes rather than osteoblasts, therefore resulting in fewer osteoblasts and lower BMD (Harslof *et al.*, 2010). In addition, PPARG activation stimulates osteoclastogenesis, therefore increasing bone resorption (Wan, 2010). Polymorphisms in the PPARG gene have been associated with BMD and implicated in the pathogenesis of osteoporosis and non-traumatic fracture risk in humans (Dragojevic *et al.*, 2011; Harslof *et al.*, 2010; Kiel *et al.*, 2007). An interaction between PPARG and dietary fat intake on BMD in mice and humans has also been suggested (Ackert-Bicknell *et al.*, 2008). In the present study, the close proximity of associated SNPs to the PPARG gene suggests that this gene may be implicated in the pathogenesis of stress fractures in racing Greyhounds, and justifies further investigation. A validation study involving further fine-mapping of the region and subsequent targeted sequencing would be required in order to confirm an association.

The eighth, ninth, tenth and eleventh most significant SNPs were BICF2S23433818, BICF2G630142608, BICF2P229977 and BICF2G63089383, respectively.

BICF2S23433818 is located on chromosome 27 (Figure 5.6) in an intergenic region between GXYLT1 (glucoside xylosyltransferase 1) and PDZRN4 (PDZ domain containing ring finger 4). BICF2G630142608 is also located on chromosome 27, in an intergenic region between PDZRN4 and CNTN1 (contactin 1). BICF2P229977 and BICF2G63089383 are both situated on chromosome 22 within the MYO16 (Myosin XVI) gene. None of the genes or proteins associated with these SNPs are known to have an involvement with bone metabolism.

A number of additional SNPs of interest were identified at the $p \leq 0.001$ significance level. The twelfth most statistically significant SNP was BICF2P252391, located on the X chromosome in an intergenic region between two uncharacterised protein-coding genes ENSCAFG00000018646 and ENSCAFG00000018649. The thirteenth most significant SNP was BICF2P647060, located on chromosome 15 within the RNF175 (ring finger protein 175) gene and just upstream of the protein-coding gene SFRP2 (Canis lupus familiaris secreted frizzled-related protein 2). SFRPs play a key role in regulating cell growth and differentiation in specific cell types. They function as soluble modulators of Wnt signalling pathways, which are signal transduction pathways involved in embryonic development and formation of important tissues, including bone. SFRP2 is expressed in bone marrow osteoblasts and independently inhibits both Wnt and bone morphogenic protein (BMP) signalling pathways, negatively affecting bone formation (Baron and Kneissel, 2013; Nakajima *et al.*, 2009). SFRP2-expressing mesenchymal stem cells (MSCs) display an increased rate of proliferation, and SFRP2 has been found to decrease MSC apoptosis and inhibit osteogenic and chondrogenic lineage commitment (Alfaro *et al.*, 2010).

The fourteenth most significant SNP was BICF2S23110295, situated on chromosome 28 in an intergenic region between TCERG1L (transcription elongation regulator 1-like) and PPP2R2D (protein phosphatase 2, regulatory subunit B, delta). Protein serine/threonine phosphatases catalyse the removal of phosphate groups from serine and/or threonine residues through hydrolysis of phosphoric acid monoesters, and play a key role in many signal transduction pathways. PPP2R2D is the B regulatory subunit of protein phosphatase 2A (PP2A), which plays a central role in the cell cycle by controlling mitosis entry and exit, with B regulatory subunits modulating substrate selectivity and catalytic activity. PPP2R2D has been identified as an important negative modulator of the TGF- β (transforming growth factor beta) signalling pathway, by restricting receptor activity (Batut *et al.*, 2008). Large quantities of TGF- β and target cells for TGF- β can be found in bone and cartilage, with autocrine and paracrine stimulation by TGF- β playing a key role in the maintenance and development of the progenitors of osteoblasts and bone formation (Chen *et al.*, 2012). Interestingly, differential expression of the PPP2R2D gene has been reported to be strongly correlated ($r^2 = 0.85$) with femur bone strength in rats, as confirmed by quantitative real-time PCR (qPCR) (Alam *et al.*, 2009).

The eighteenth most significant SNP was BICF2P381996 located on chromosome 17 within the ATP6V1C2 (ATPase, H⁺ transporting, lysosomal 42kDa, V1 subunit C2) gene, encoding a component of vacuolar ATPase (V-ATPase). V-ATPase is an enzyme transporter responsible for acidification of intracellular compartments in

eukaryotic cells, and plays a vital role in osteoclastic bone resorption. As a result of mutations or deletions in the genes encoding V-ATPase subunits, proton secretion from the ruffled border membrane of the osteoclast does not occur and therefore resorptive activity in the bone is decreased (Qin *et al.*, 2012). Polymorphisms within the ATP6V1C2 gene may be involved in the pathogenesis of stress fractures in racing Greyhounds, and warrant further investigation.

5.5.4. Limitations of the Study Design

A limitation of the GWA study is the small sample size of 21 case (affected) and 24 control (unaffected) Greyhounds. This increases the risk of identifying false positive associations, and potentially important SNPs may have been missed. No SNPs were found to reach genome-wide significance levels ($p \leq 0.00001$) or remained significant after Bonferroni correction, and this is likely to be due to the small sample size used in the study. Power calculations have indicated that for a multigenic trait, a minimum GWAS sample size of 100 affected and 100 unaffected dogs would be optimal (Lindblad-Toh *et al.*, 2005). It is therefore likely that the present study is underpowered, and it would be beneficial in future canine GWA studies to include larger numbers of case and control individuals.

A case-control study design for GWAS is based on the assumption that the individuals in the study population are 'unrelated', in that the maximum familial relationship between any two individuals is less than that of a second-degree relative (Anderson *et al.*, 2010). The familial relationship between any two

Greyhounds selected for the GWA study ($n = 48$), however, was often that of a second-degree relative. Including related individuals may introduce bias, as particular within-family genotypes are over-represented in the study. Consequently, this is a further limitation of the study design.

The use of popular breeding sires and AI are very common in the racing Greyhound Industry, with the top breeding sires each producing thousands of offspring. As a result, any two individual Greyhounds currently racing in the UK are likely to have at least one common ancestor between them within the previous two to three generations. Full pedigree data was obtained for each racing Greyhound in the present study and familial relationships between the dogs were considered when selecting samples to include in the GWAS, in order to ensure that all individuals were as unrelated as possible. This study design, however, is not ideal for a case-control association study. A family-based design may be more appropriate for future racing Greyhound GWA studies, as this would control for heterogeneity and population stratification. A family-based approach, however, would require first-degree relatives for analysis. Consequently, obtaining sufficient DNA samples for the study may be difficult, particularly for within-family case (affected) dogs.

In the present study, case and control Greyhounds were selected from the same population, and this is an important criterion for sample selection in GWA studies. In order to obtain sufficient samples for the study, further matching of case and control individuals was not possible. Additionally, the inclusion criteria of 50 races or less for case Greyhounds and over 70 races for control Greyhounds may have

resulted in two different age groups for case and control individuals. An advantage of the present study design, however, was that all Greyhounds were extremely well phenotyped, with detailed pedigree, phenotypic, race history and injury data obtained for both case and control individuals. Previously reported canine GWA studies have often made use of control DNA samples obtained from DNA archives, with no case-control matching beyond population and usually with very little pedigree, phenotypic data or history available for the individual animal (Massey *et al.*, 2014; Wood *et al.*, 2009).

5.5.5. Conclusions

In conclusion, the present study is the first investigation of genetic factors in the pathogenesis of stress fracture injuries in the racing Greyhound. No SNPs were found to reach genome-wide statistical significance at the $p \leq 0.00001$ significance level, or remained significant after Bonferroni correction, and this is likely to be due to the small sample size used in the study. As an initial discovery GWAS, however, the study has identified several nominally associated SNPs and regions of interest on chromosomes that may be implicated in the pathogenesis of stress fractures in the racing Greyhound. 11 SNPs were significantly associated at the $p \leq 0.0001$ level and a further 96 SNPs were significantly associated at the $p \leq 0.001$ level. Polymorphisms identified within or in close proximity to the PPARG, SFRP2, PPP2R2D and ATP6V1C2 genes are of particular interest, due to the function and involvement of these genes in bone metabolism pathways. The next stage would be to confirm the associations by fine-mapping in a validation study, using a larger

cohort of cases and controls from the same Greyhound population, and subsequent targeted sequencing of the identified regions of interest.

The present study has therefore identified several candidate genes for hypothesis-driven further research into genetic factors associated with stress fracture injuries in the racing Greyhound. It is hoped that through further work in this area, associations with the disease phenotype will be confirmed and specific genetic risk factors identified. Eventually, this could be used to inform the selection of racing Greyhounds for breeding, in order to improve Greyhound welfare by producing stronger racing animals that are less predisposed to injury.

Chapter Six.

Salivary Cortisol and Heart Rate as Measures of Stress in the Racing Greyhound

6. Salivary Cortisol and Heart Rate as Measures of Stress in the Racing Greyhound

6.1. Introduction

6.1.1. Cortisol as a Measure of Stress

There is no one clear definition of stress. The most commonly used terminology, however, define environmental stimuli that result in an imbalance of homeostasis as 'stressors', and the subsequent physiological reaction of the individual as a 'stress response' (Mostl and Palme, 2002). As a physiological reaction, stress in animals may be considered as negative arousal, for example due to fear, or as positive arousal, for example due to excitement, increased alertness and preparation for activity (Broom and Johnson, 1993; Dobson and Smith, 1995; Matteri *et al.*, 2000; Mostl and Palme, 2002; Pastore *et al.*, 2011; Raynaert *et al.*, 1976).

Cortisol is a glucocorticoid hormone secreted by the adrenal gland due to activation of the HPA axis in response to external and internal stimuli (Matteri *et al.*, 2000; Mostl and Palme, 2002). It is a frequently used measure of physiological stress in most mammals, including humans (Stawski *et al.*, 2013), dogs (Beerda *et al.*, 1996, 1998; Hekman *et al.*, 2012; Kobelt *et al.*, 2003; Pastore *et al.*, 2011), horses (Fazio *et al.*, 2008a,b, 2013; Schmidt *et al.*, 2010a,b,c,d), sheep (Hargreaves and Hutson, 1990), cattle and pigs (Von Borell *et al.*, 2007). Cortisol is commonly used as a

marker for assessment of canine welfare (Menor-Campos *et al.*, 2011), reaction to stress challenges (Bergeron *et al.*, 2002) and human-animal interaction studies (Bergamasco *et al.*, 2010; Jones and Josephs, 2006), and has been shown to be a useful measure of acute and chronic physiological and psychological stress in dogs.

Glucocorticoid hormones such as cortisol are known to affect bone physiology, both directly and indirectly, resulting in reduced activity and apoptosis of osteoblasts and increased activity of osteoclasts (Hardy and Cooper, 2010). High circulating levels of glucocorticoids are associated with an increased risk of osteoporosis and bone fracture in humans (Hardy and Cooper, 2010).

Previous studies have investigated the effect of exercise on cortisol levels in agility and sled dogs (Angle *et al.*, 2009; Pastore *et al.*, 2011; Rovira *et al.*, 2007; Wakshlag *et al.*, 2004), and in equine (Kedzierski *et al.*, 2013, 2014) and human (Dittrich *et al.*, 2013) athletes. The effects of exercise and racing on cortisol concentrations in Greyhounds, however, have not previously been reported.

It is hypothesised that racing is a stressful stimulus for Greyhounds that results in activation of the HPA axis and increased secretion of cortisol, as well as increased activity of the autonomic nervous system resulting in elevation of HR. Furthermore, it is hypothesised that the stress response of the Greyhound, as measured by salivary cortisol concentration and HR, is associated with Greyhound gender, age, journey distance to the race track and performance in the race.

6.1.2. Measurement of Canine Cortisol

Canine cortisol levels can be measured in a wide range of biological samples including blood (Angle *et al.*, 2009; Bergeron *et al.*, 2002; Hennessy *et al.*, 1998; Vincent and Michell, 1992); saliva (Beerda *et al.*, 1998; Bergeron *et al.*, 2002; Dreschel and Granger, 2009; Hekman *et al.*, 2012; Kobelt *et al.*, 2003; Vincent and Michell, 1992); hair (Accorsi *et al.*, 2008; Bryan *et al.*, 2013; Ouschan *et al.*, 2013); urine (Beerda *et al.*, 1996) and faeces (Accorsi *et al.*, 2008). Plasma cortisol levels most accurately reflect the activity of the HPA system. Blood sampling, however, is considered an invasive procedure, and a home office licence is required for collection of blood samples from live dogs for research purposes in the UK. Owners and trainers of Greyhounds may be reluctant to consent to invasive collection of a blood sample from their dogs, particularly before or after racing. Additionally, venipuncture and the handling and restraint required for the procedure may be stressful for the animal, and have been found to increase canine blood cortisol levels 20 minutes later (Hennessy *et al.*, 1998).

Saliva collection is considered a non-invasive procedure and does not require a licence. Canine salivary cortisol concentrations are highly correlated with plasma cortisol (Beerda *et al.*, 1996; Vincent and Michell, 1992), and can therefore be used as a measure of HPA activity. Vincent and Michell (1992) suggested that there was evidence of a delay in some dogs between changes in plasma and salivary cortisol concentrations. Beerda *et al.* (1996), however, found no delay between changes in salivary cortisol levels relative to plasma.

Salivary cortisol represents unbound, free cortisol whereas plasma cortisol is mainly bound to carrier proteins (Mostl and Palme, 2002). Salivary cortisol concentrations are therefore lower relative to plasma, with reported salivary cortisol concentrations between 4% and 11.9% of comparable plasma values (Beerda *et al.*, 1996; Vincent and Michell, 1992). Of the total cortisol fraction in plasma, only the small, highly lipid-soluble molecules of the unbound fraction transfer through cell membranes into saliva (Beerda *et al.*, 1996).

Although handling of the animal is still required to obtain a saliva sample, Kobelt *et al.* (2003) reported that a saliva sample could be collected from a dog for up to four minutes without the process of handling affecting cortisol measurements.

6.1.3. Transport of Greyhounds to the Race Track

Racing Greyhounds are exposed to several different stressors during race meetings, such as transport of the Greyhound to the race track, pre-race veterinary inspections, kennelling, noise from spectators and the actual race itself.

British Greyhounds will typically compete in one race per week, with additional training and trial sessions. There are 26 GBGB-licensed Greyhound stadiums and a further nine Independent race tracks in the UK to date, with Greyhound trainers and racing kennels situated across the UK. Consequently, there is considerable variation in the distance routinely travelled to the race track for attendance at race

and trial meetings. Open Race Greyhounds will often travel long distances to Greyhound tracks across the UK in order to compete. The GBGB provide guidelines for the transport of racing Greyhounds within the UK (Greyhound Board of Great Britain, 2014). Specifically, the guidelines state that the journey time from the kennels to race track should be less than eight hours; a maximum of two Greyhounds should be transported loose in the back of a motor vehicle; if more than two Greyhounds are to be transported, then each should be held in a separate travel cage with minimum dimensions of 35.56 cm wide, 101.6 cm length and 76.2 cm height (larger cage sizes are required for journeys greater than eight hours); and Greyhounds should not be loaded for transport for at least 15 to 30 minutes after racing or trialling to allow them to cool. These guidelines do not apply to the Independent sector of the Greyhound industry. The transport of all live vertebrate animals within the European Union that takes place in connection with an economic activity such as Greyhound racing, however, must comply with the Council Regulation (EC) No 1/2005 on the protection of animals during transport and related operations.

Travelling in a motor vehicle is recognised as a stressful event for horses (Fazio *et al.*, 2008a,b, 2013; Kay and Hall, 2009; Medica *et al.*, 2010; Schmidt *et al.*, 2010a,b,d) and dogs (Bergeron *et al.*, 2002), based on increases in cortisol concentrations following road transport. Additionally, it has been suggested that cortisol levels are positively correlated with transport time (Fazio and Ferlazzo, 2003). Racing Greyhounds are frequently transported by road for race, trial and

sale events, however the effect of transport on Greyhound cortisol levels has not previously been reported.

Changes in HR are frequently used as an additional marker of psychophysiological stress in livestock animals and horses (Bergamasco *et al.*, 2010). Transport of horses by road has been reported to cause changes in HR and heart rate variability that are indicative of stress (Schmidt *et al.*, 2010a,b,d). The effects of road transport and racing on the HR of racing Greyhounds, however, have not previously been reported.

6.2. Aims

The study aimed to investigate measures of the stress response to short-duration high-intensity sprint exercise activity in British Greyhounds, by measurement of salivary cortisol concentration and HR immediately pre- and post-racing at an Independent (non-GBGB licensed) stadium. In addition, the study aimed to consider the effects of journey distance to the stadium, Greyhound sex, racing age, distance of the race and rank finish position on pre- and post-race cortisol concentration and HR.

6.3. Materials and Methods

6.3.1. Sample and Data Collection

Greyhounds were recruited to take part in the study as detailed in Chapter 2.3.1. A total of 59 non-GBGB licensed Greyhounds (32 males and 27 females) were included in the study, with ages ranging from 16 to 84 months (mean age of 37.25 months). The rules and regulations of the GBGB restrict the collection of samples from Greyhounds present at licensed race track premises. Consequently, sampling took place at one Independent (non-GBGB-licensed) Greyhound stadium in the North-West of the UK. Samples were collected over two consecutive race meetings held one week apart, in December 2013. Six of the Greyhounds (four males and two females) were sampled on two separate occasions, at both the first and second race meetings.

Pre-race and post-race saliva samples were collected from Greyhounds using SCS (Salimetrics® Europe Limited, Suffolk, UK), as detailed in Chapter 2.3.2.1. A total of 65 pre-race and 63 post-race saliva samples were collected over the two consecutive race meetings, providing 128 saliva samples for analysis. 80 of the saliva samples were collected during the first race meeting, and 52 were collected during the second race meeting.

Phenotypic data, and pre-race and post-race HR measurements (Appendix 10), were collected from Greyhounds as detailed in Chapter 2.3.2. A total of 65 pre-race and 58 post-race HR measurements were collected over the two consecutive race meetings, providing 123 HR measurements for analysis. 73 of the HR measurements were collected during the first race meeting, and 50 were collected during the second race meeting.

The differences between numbers of pre-race and post-race HR measurements and saliva samples were due to several Greyhound owners or trainers declining post-race heart rate measurement and/or saliva sampling of the dog.

6.3.2. Cortisol Enzyme Immunoassay (EIA)

The saliva samples were processed as detailed in Chapter 2.3.3. A total of 16 samples (all pre-race) contained an insufficient quantity of saliva for cortisol measurement, and were therefore excluded from further analysis. Cortisol concentration was measured in the remaining 106 saliva samples using an EIA as detailed in Chapter 2.3.3, at the Department of Biological Sciences at the University of Chester (Chester, UK) (R. Coleman). The cortisol concentration in one of the post-race saliva samples was too low to measure. Consequently, cortisol was successfully measured in a total of 111 saliva samples (49 pre-race and 62 post-race samples) (Appendix 10).

6.3.3. Statistical Analysis

Statistical analysis and production of graphical representations of the data were performed using the software packages SAS, version 9.3 (SAS Institute Inc., 2002-2010) and Microsoft Office Excel (Microsoft Corporation, 2007b).

Paired t-tests were performed in Microsoft Office Excel (Microsoft Corporation, 2007b) in order to investigate differences between each Greyhound's paired salivary cortisol concentration pre- and post-racing, and between each Greyhound's paired HR pre- and post-racing. Using ANOVA analyses, the data was examined for associations between pre- or post-race salivary cortisol concentration or HR and each of the following factors: Greyhound sex (male or female); distance of the race (265 m, 420 m or 470 m); and adjusted rank finish position (1 to 6). Scatter plots were produced in order to investigate the association between salivary cortisol concentration and HR, both pre- and post-race. Similarly, the data was examined for associations between pre- or post-race salivary cortisol concentration or HR and each of the following factors: journey distance of the Greyhound to the race track (1.61 km to 137.28 km), and age of the Greyhound (16 months to 84 months).

6.4. Results

6.4.1. Pre- and Post-Race Salivary Cortisol Concentration

Overall, pre-race salivary cortisol concentration ($n = 49$) ranged from 0.36 nanograms per millilitre (ng/ml) to 50.89 ng/ml, with a mean of 12.17 ng/ml and SD of 10.95 (SE 1.56). Post-race salivary cortisol concentration ($n = 62$) ranged from 2.10 ng/ml to 92.74 ng/ml, with a mean of 21.71 ng/ml and SD of 15.63 (SE 2.00). The distributions of both pre- and post-race salivary cortisol concentration are displayed in Figure 6.1. For the paired samples, where each Greyhound had both a pre-race and post-race salivary cortisol measurement ($n = 47$), the pre-race salivary cortisol ranged from 0.36 ng/ml to 50.89 ng/ml and post-race ranged from 2.10 ng/ml to 92.74 ng/ml. The mean pre-race cortisol concentration was 12.16 ng/ml with a SD of 11.18 (SE 1.63), and the mean post-race was 23.08 ng/ml with a SD of 16.48 (SE 2.40).

The pre-race and post-race salivary cortisol data were logged in order to normalise the distributions for further analysis. The distributions of pre-race ($n = 49$), post-race ($n = 62$), and both pre- and post-race salivary log cortisol concentration are displayed in Figure 6.2, Figure 6.3 and Figure 6.4, respectively. The paired pre-race and post-race log cortisol measurements for each individual ($n = 47$) are displayed in Figure 6.5. The differences between each individual's pre-race and post-race values are displayed in Figure 6.6. There was a significant difference ($p = 0.000004$;

test statistic = 5.26) between each Greyhound's paired pre-race and post-race salivary log cortisol concentration. Post-race values were significantly higher than pre-race values, with a mean difference of 0.78 ng/ml and SD 1.02 (SE 0.15).

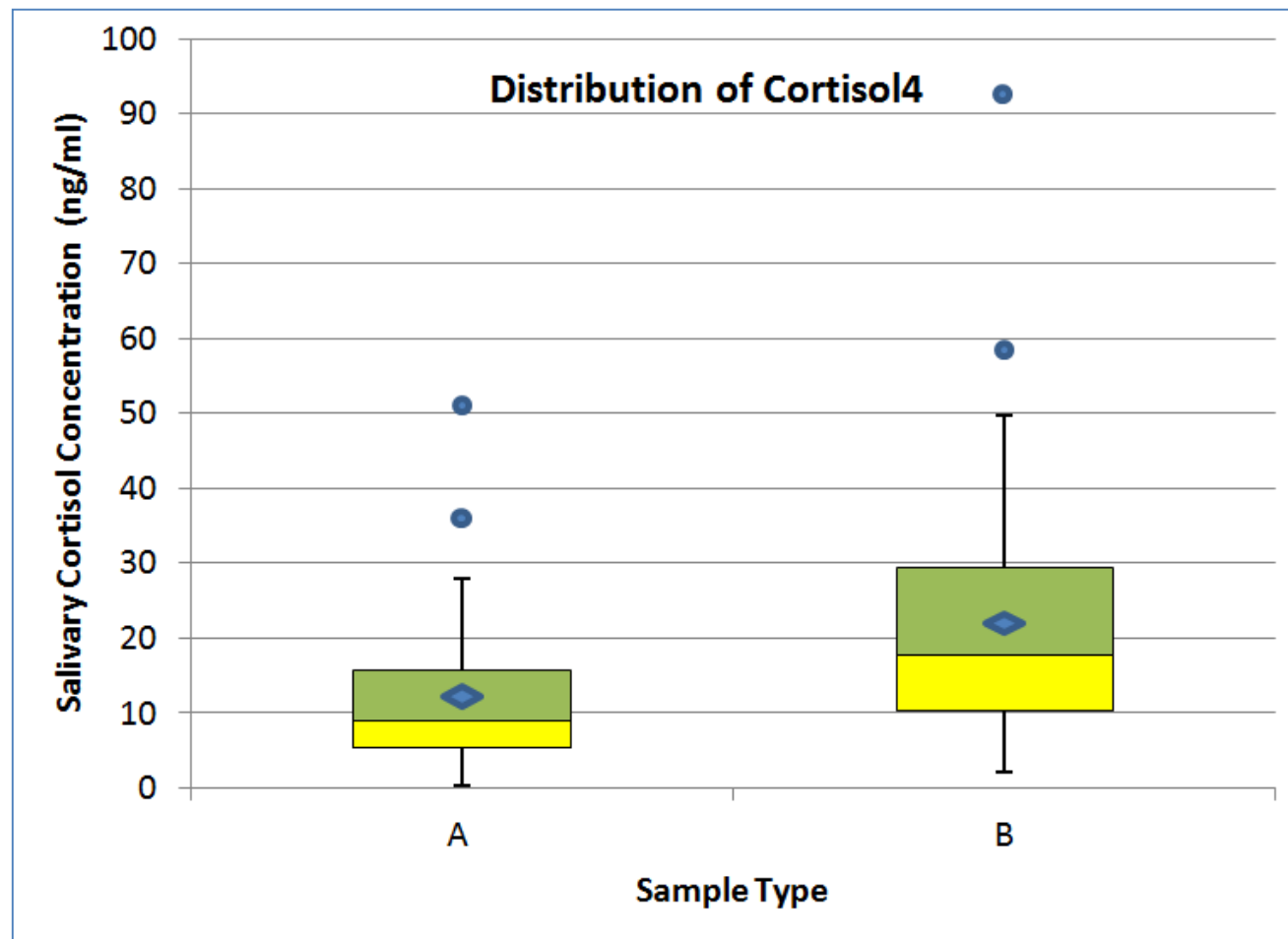


Figure 6.1. The distribution of pre-race (A) (n = 49) and post-race (B) (n = 62) Greyhound salivary cortisol concentration (ng/ml).

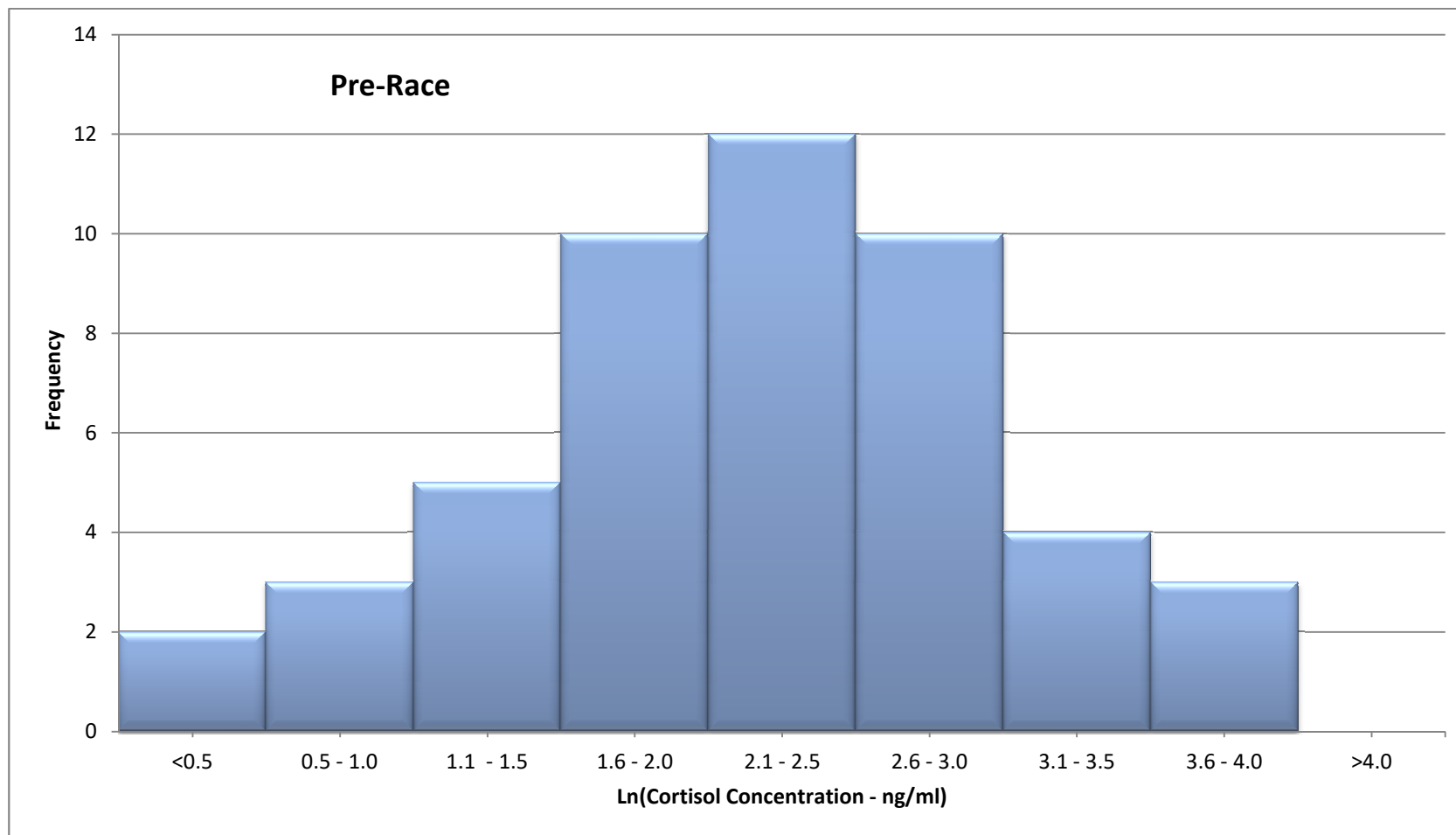


Figure 6.2. The distribution of Greyhound salivary log cortisol concentration (ng/ml) measured pre-race (n = 49).

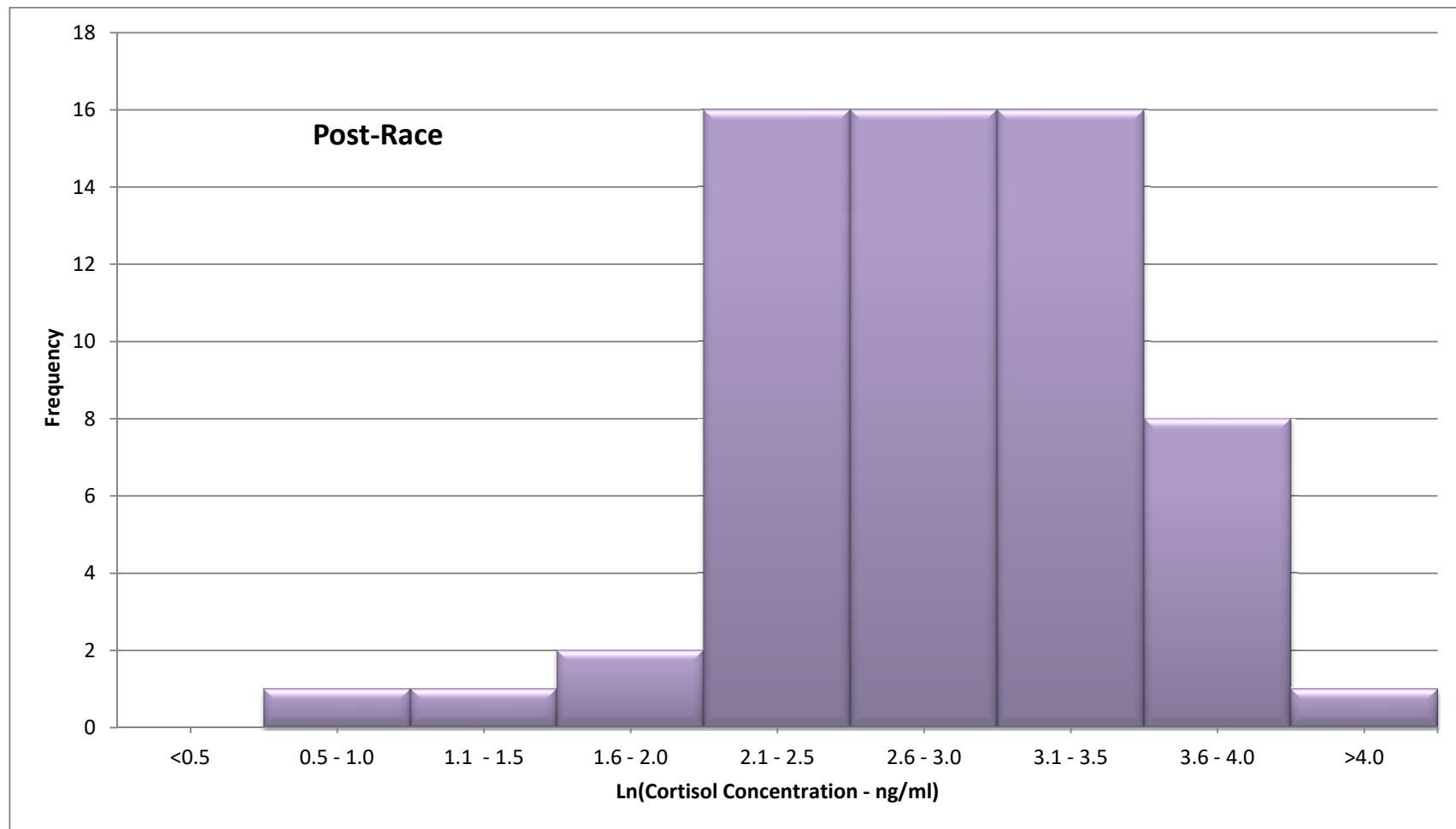


Figure 6.3. The distribution of Greyhound salivary log cortisol concentration (ng/ml) measured post-race (n = 62).

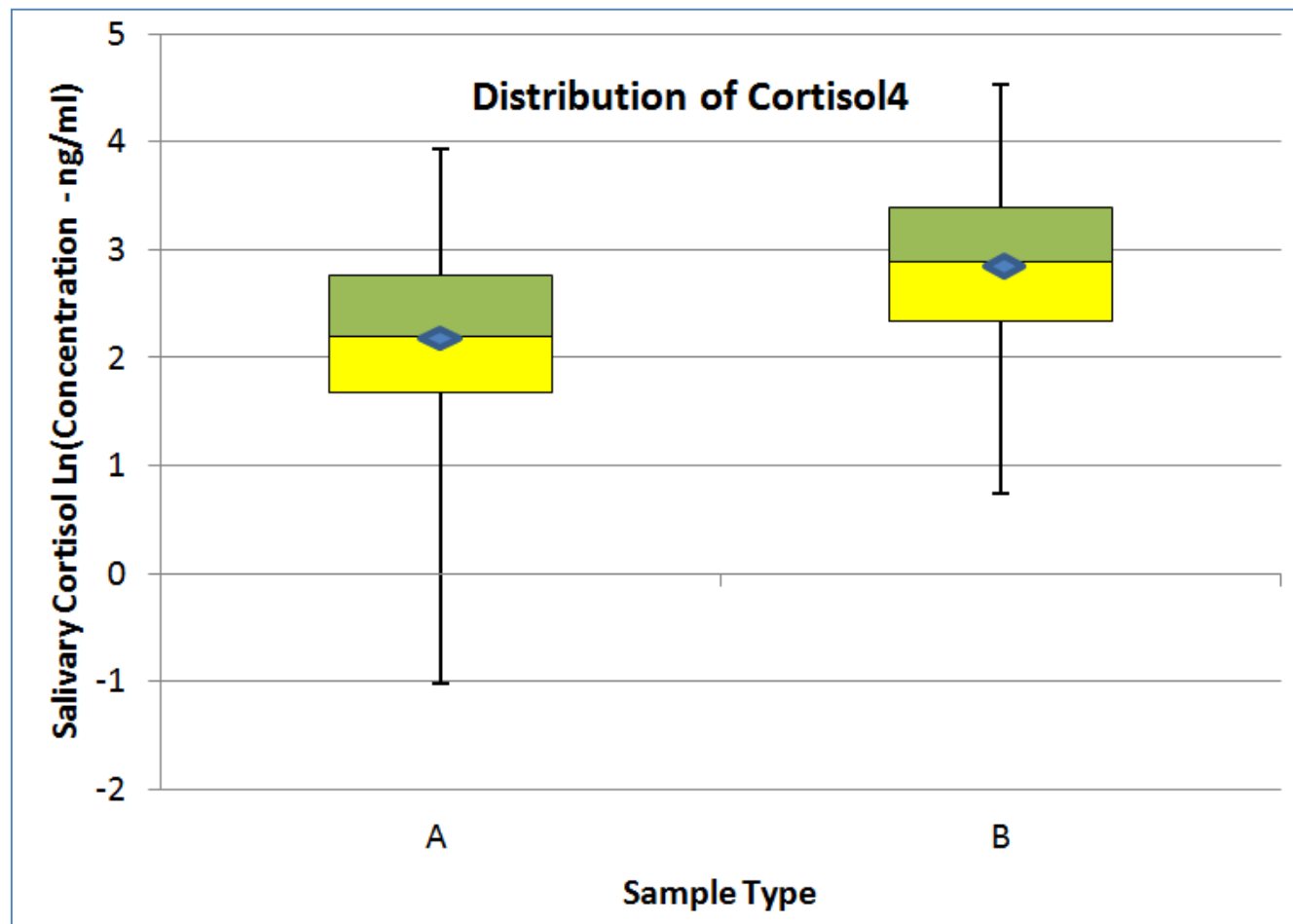


Figure 6.4. The distribution of pre-race (A) (n = 49) and post-race (B) (n = 62) Greyhound salivary log cortisol concentration (ng/ml).

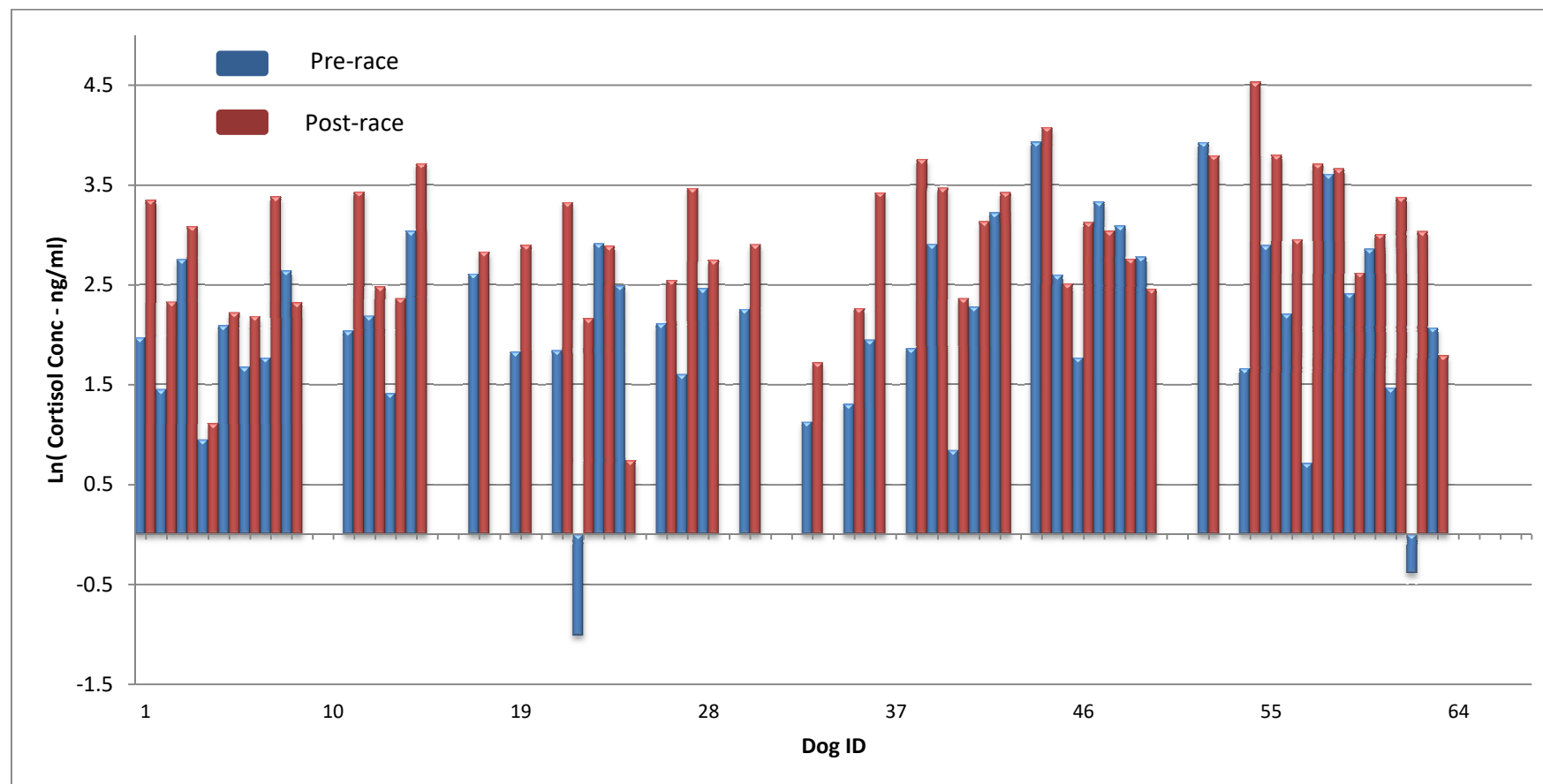


Figure 6.5. The paired pre-race and post-race salivary log cortisol concentration (ng/ml) for each Greyhound ID (1 to 67) (n = 47).

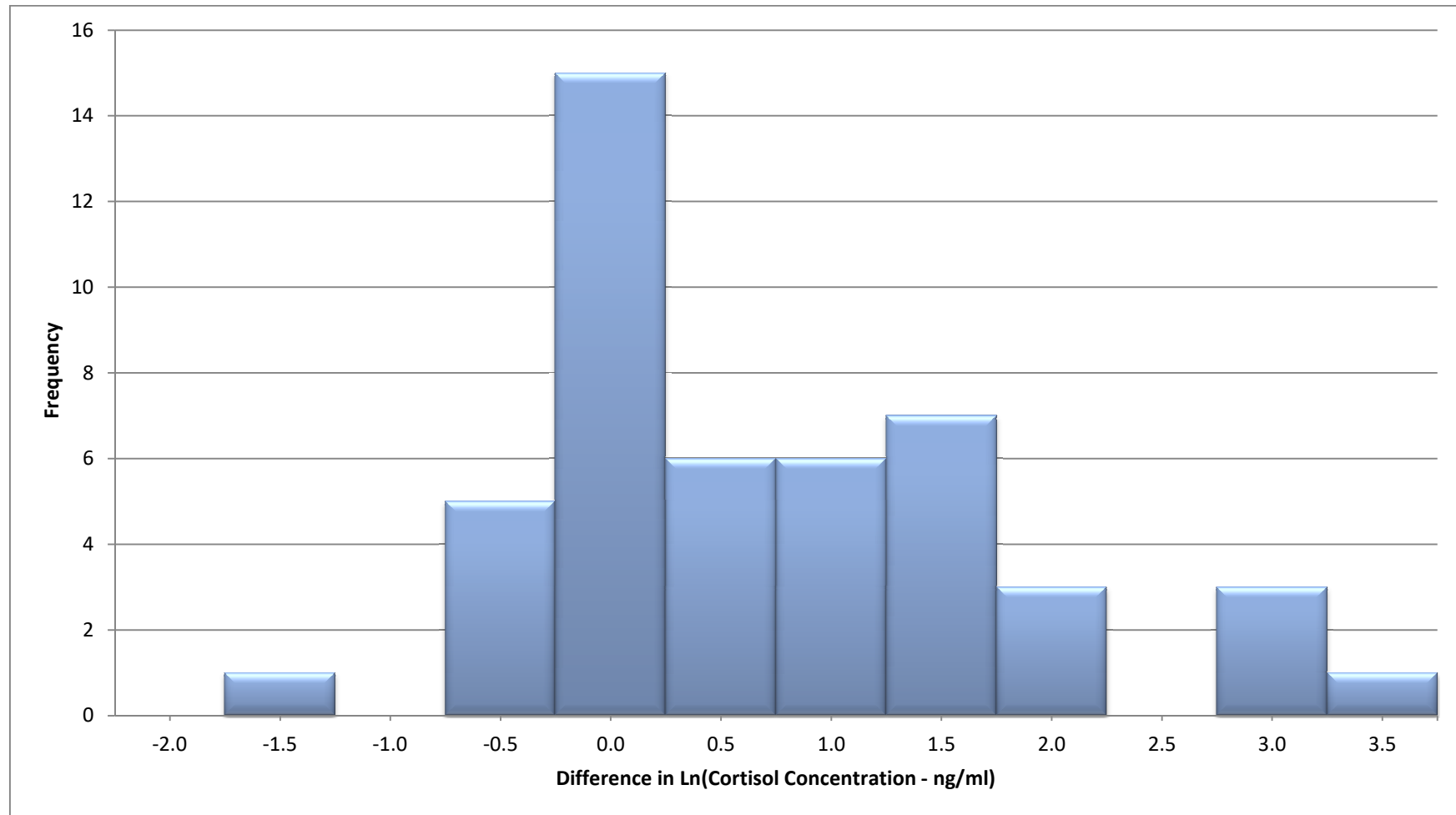


Figure 6.6. Difference in salivary log cortisol concentration (ng/ml) between pre- and post-race paired samples for each Greyhound (n = 47).

6.4.2. Pre- and Post-Race Heart Rates

Overall, pre-race HR ($n = 65$) ranged from 84 beats per minute (bpm) to 240 bpm, with a mean of 155.14 bpm and SD of 32.90 (SE 4.05). Post-race HR ($n = 58$) ranged from 96 bpm to 240 bpm, with a mean of 175.03 bpm and SD of 36.43 (SE 4.78). The distributions of pre-race HR, post-race HR, and both pre- and post-race HR are displayed in Figure 6.7, Figure 6.8 and Figure 6.9, respectively.

For the paired samples, where each Greyhound had both a pre-race and post-race HR measurement ($n = 58$), the pre-race HR ranged from 84 bpm to 240 bpm and post-race ranged from 96 bpm to 240 bpm. The mean HR pre-race was 155.31 bpm with a SD of 34.04 (SE 4.46), and the mean post-race was 175.03 bpm with a SD of 36.43 (SE 4.78). The paired pre-race and post-race HR measurements for each individual ($n = 58$) are displayed in Figure 6.10. The differences between each individual's pre-race and post-race values are displayed in Figure 6.11. There was a significant difference ($p = 0.002$; test statistic = 3.24) between each Greyhound's paired pre-race and post-race HR. Post-race values were significantly higher than pre-race values, with a mean difference of 19.72 bpm and SD of 46.29 (SE 6.08).

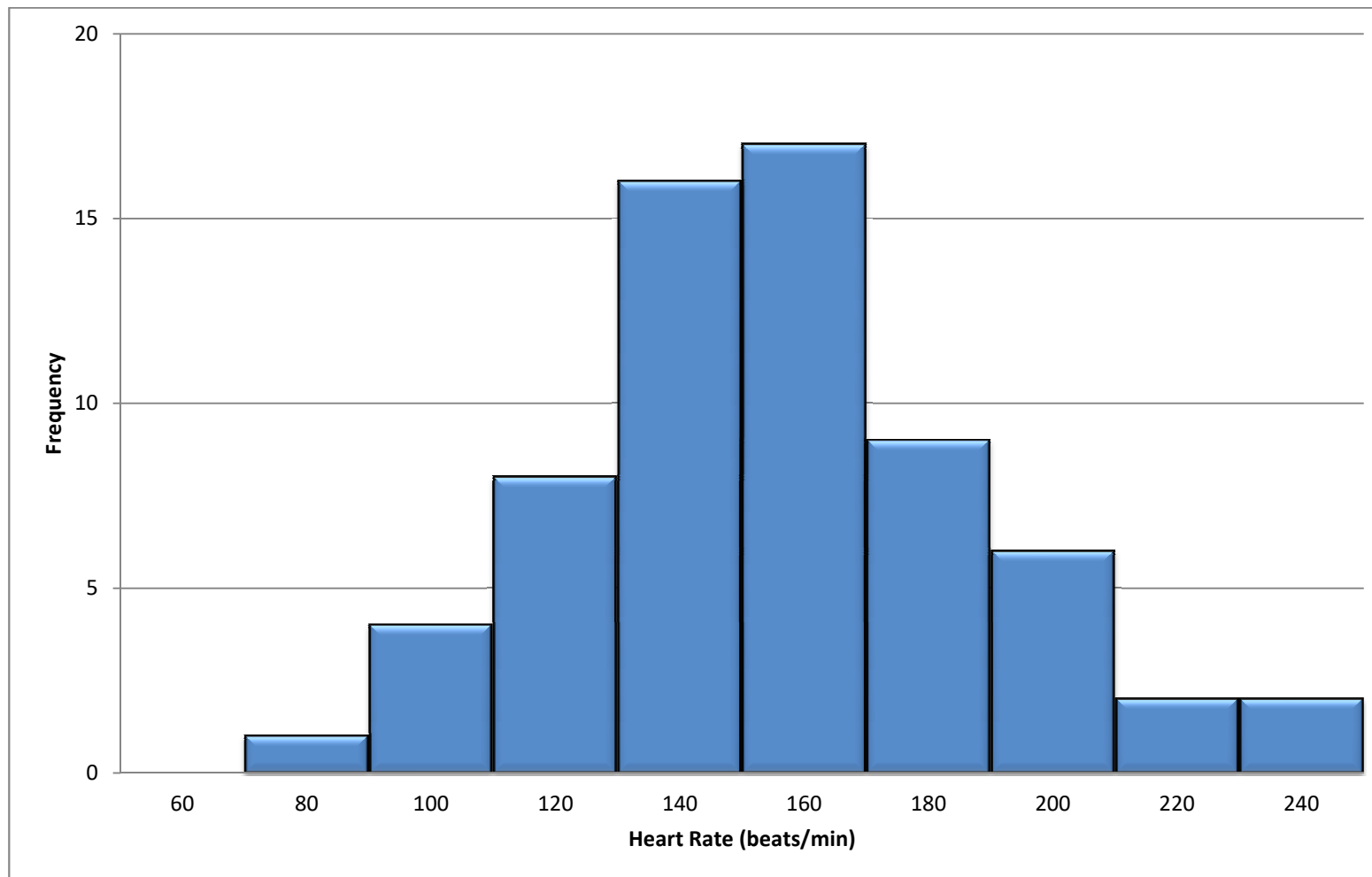


Figure 6.7. The distribution of Greyhound heart rate (beats per minute) measured pre-race (n = 65).

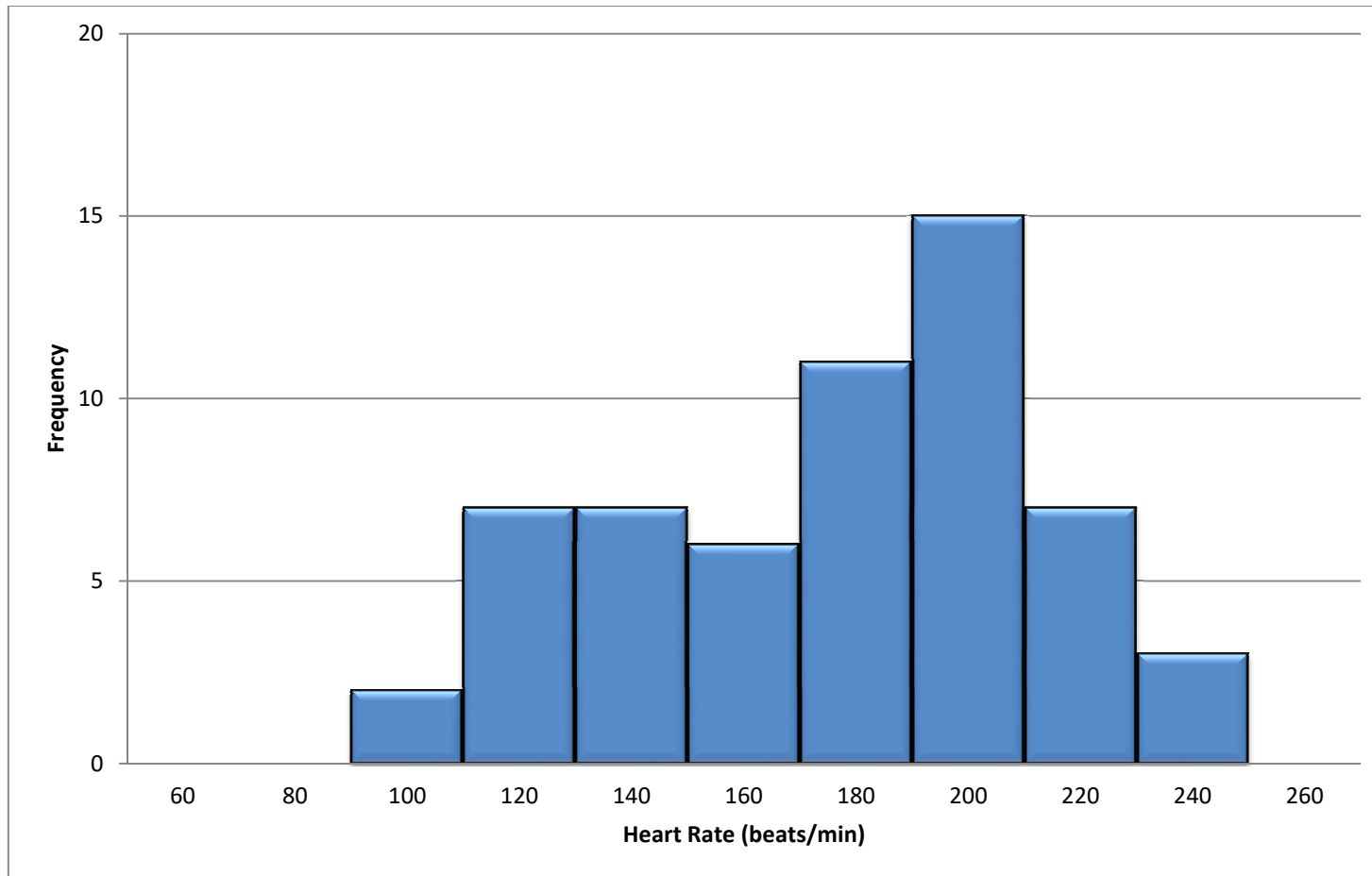


Figure 6.8. The distribution of Greyhound heart rate (beats per minute) measured post-race (n = 58).

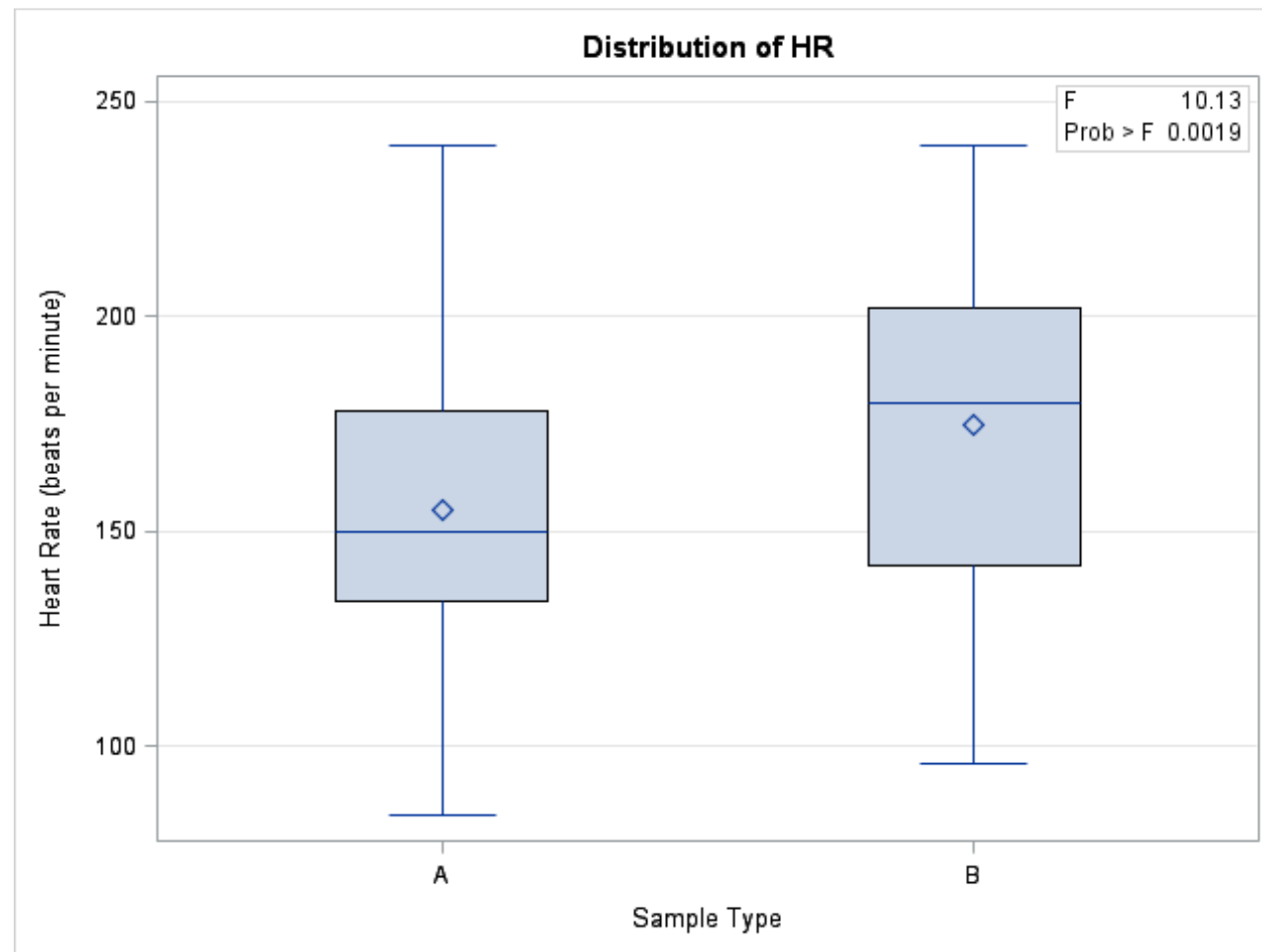


Figure 6.9. The distribution of pre-race (A) (n = 65) and post-race (B) (n = 58) Greyhound heart rate (beats per minute).

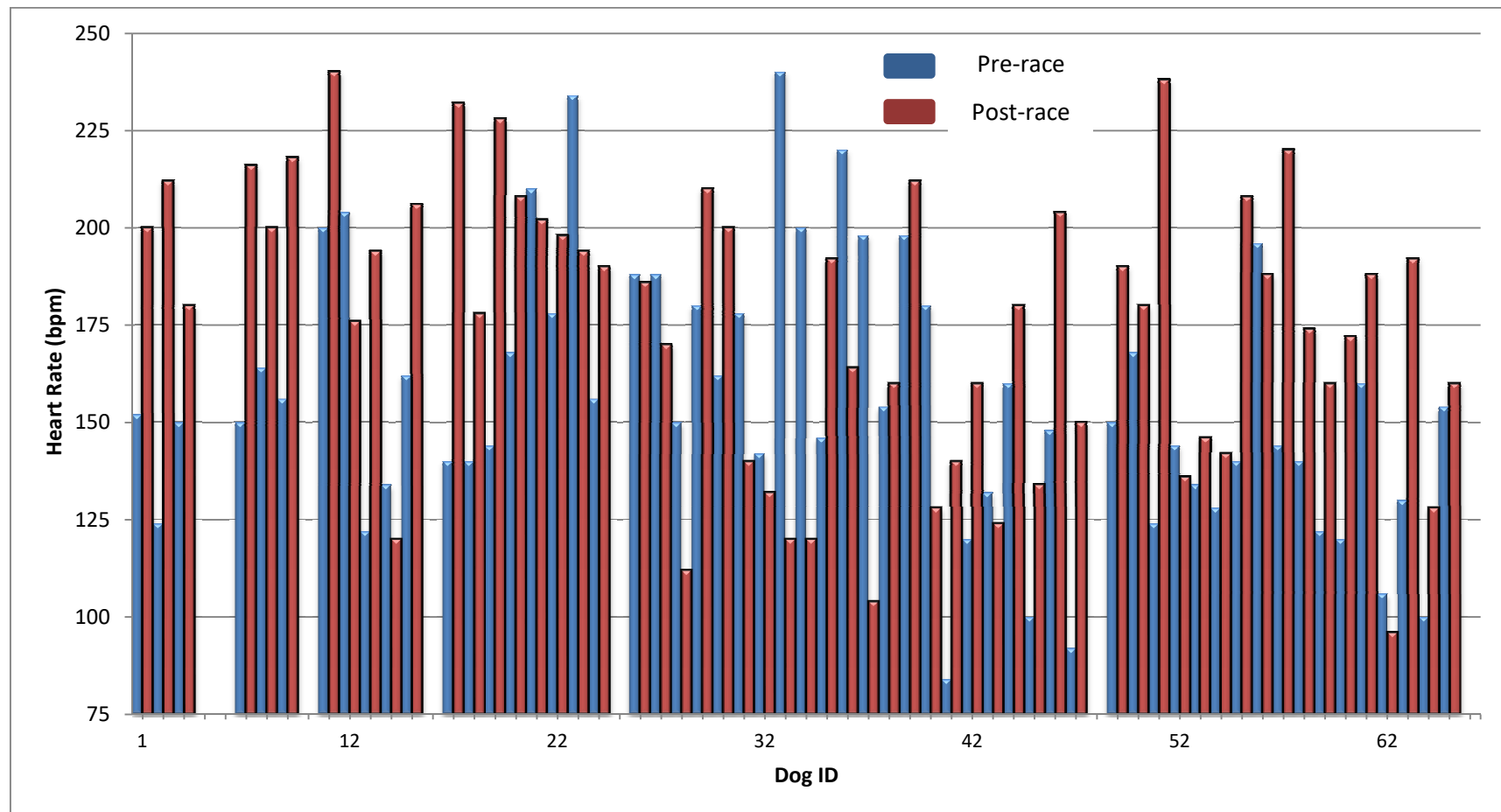


Figure 6.10. Paired pre-race and post-race heart rate (beats per minute) for each Greyhound ID (1 to 67) (n = 58).

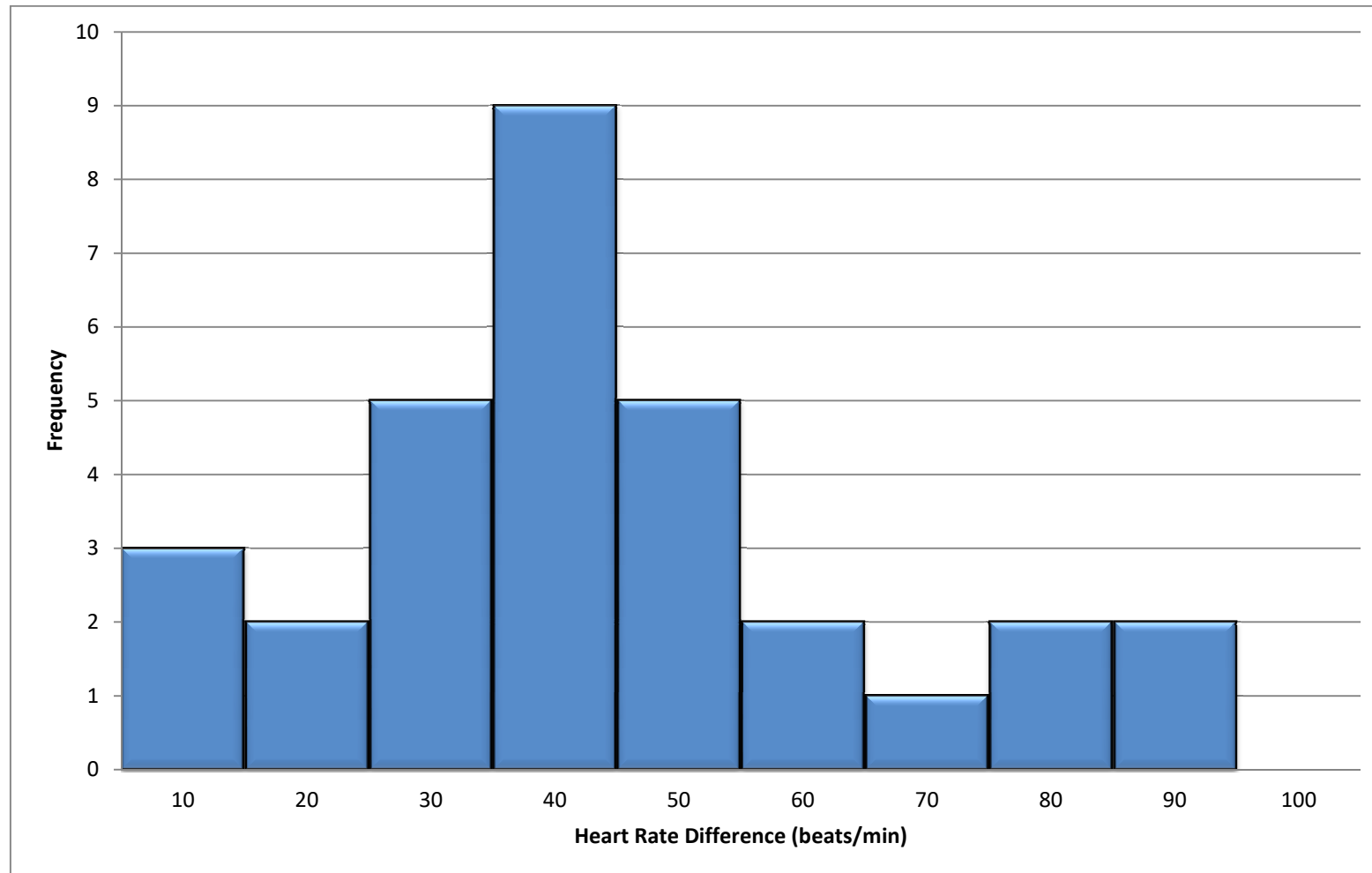


Figure 6.11. Heart Rate difference (beats per minute) between pre- and post-race paired measurements for each Greyhound (n = 58).

6.4.3. Correlation Between Salivary Log Cortisol Concentration and Heart Rate

No correlations were found between Greyhound HR and salivary log cortisol concentration in pre-race ($R^2 = 0.0098$) or post-race ($R^2 = 0.0104$) measurements, as displayed in Figure 6.12 and Figure 6.13, respectively.

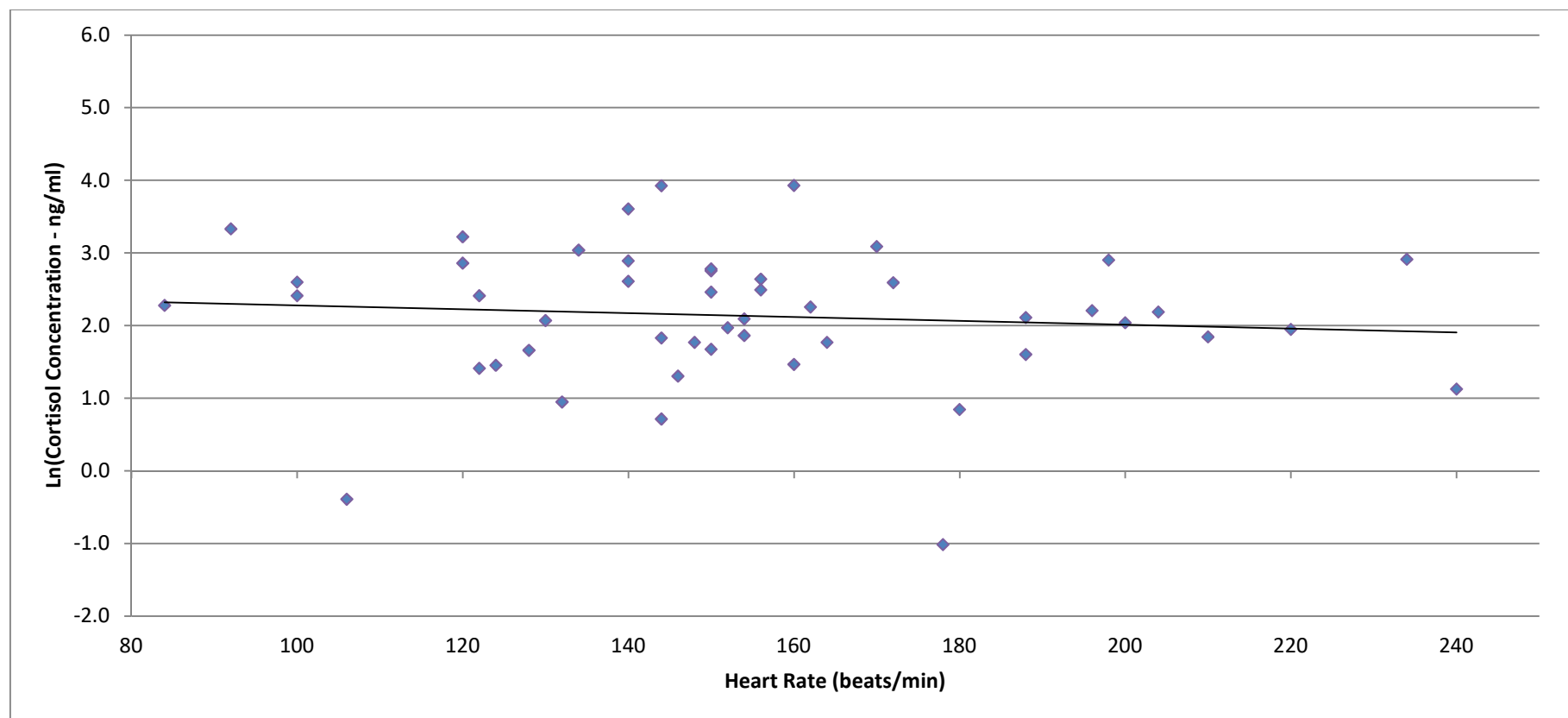


Figure 6.12. Scatter plot of Greyhound pre-race salivary log cortisol concentration (ng/ml) and heart rate (beats per minute) ($R^2 = 0.0098$).

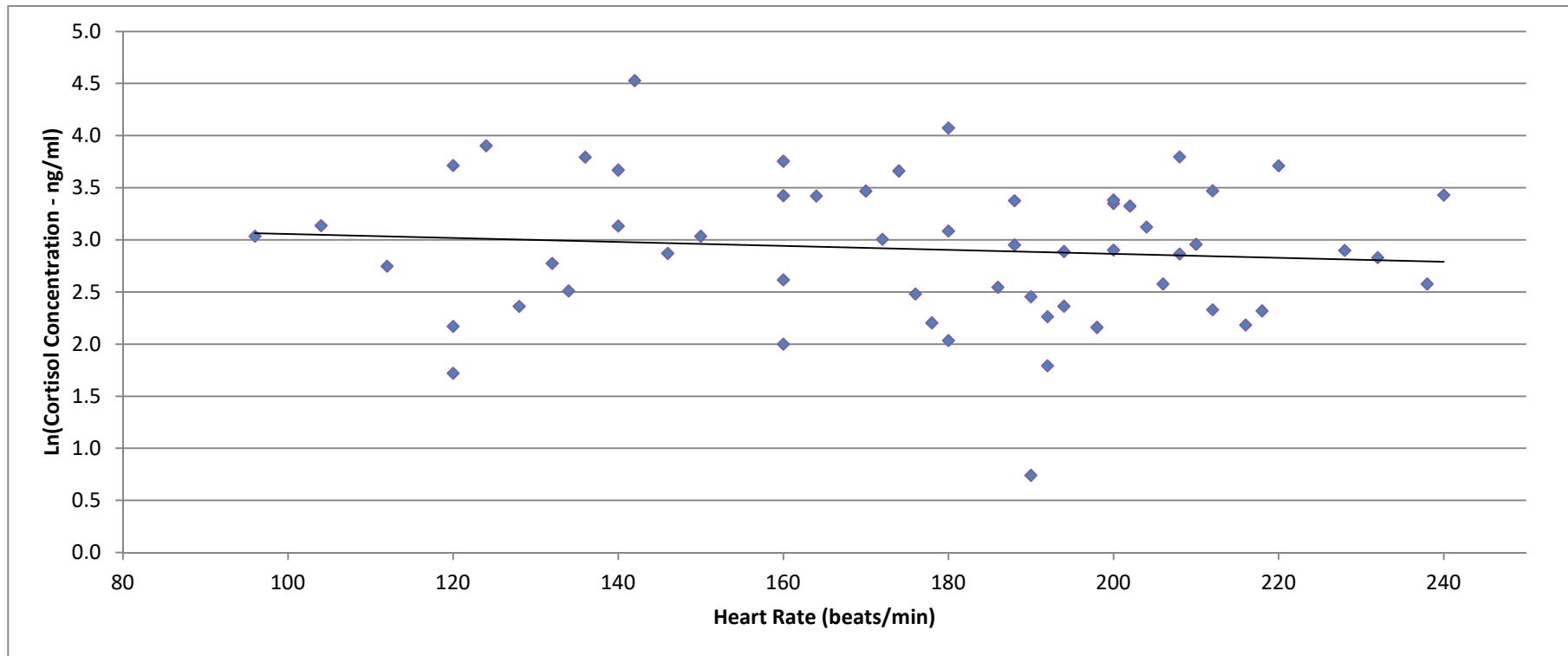


Figure 6.13. Scatter plot of Greyhound post-race salivary log cortisol concentration (ng/ml) and heart rate (beats per minute) ($R^2 = 0.0104$).

6.4.4. Influence of Environmental Factors on Pre- and Post-Race Cortisol Concentration and Heart Rate

The sex of the dog (male or female) (Figure 6.14 to Figure 6.17), race distance (265 m, 420 m or 470 m) (Figure 6.18 and Figure 6.19) and rank finish position (1 to 6) in the race (Figure 6.20 and Figure 6.21) were each found to have no significant association ($p > 0.05$) with pre-race or post-race salivary log cortisol concentration (ng/ml) or HR (bpm) (Table 6.1).

Association	Measure of Stress			
	Salivary Log Cortisol Concentration (ng/ml)		Heart Rate (bpm)	
	Pre-Race	Post-Race	Pre-Race	Post-Race
Sex	$p = 0.9995$ $F = 0.00000048$ $df (1, 47)$	$p = 0.1916$ $F = 1.7453$ $df (1, 59)$	$p = 0.7692$ $F = 0.0871$ $df (1, 47)$	$p = 0.5085$ $F = 0.4427$ $df (1, 56)$
Race Distance	$p = 0.8517$ $F = 0.1610$ $df (2, 46)$	$p = 0.1802$ $F = 1.7654$ $df (2, 58)$	$p = 0.3859$ $F = 0.9722$ $df (2, 46)$	$p = 0.8018$ $F = 0.2217$ $df (2, 56)$
Rank	$p = 0.2577$ $F = 1.3169$ $df (10, 37)$	$p = 0.2531$ $F = 1.3062$ $df (10, 50)$	$p = 0.6672$ $F = 0.7576$ $df (10, 38)$	$p = 0.9782$ $F = 0.2976$ $df (10, 45)$

Table 6.1. ANOVA results [p -values, F -statistics and degrees of freedom (between groups, within groups)] of associations between Greyhound sex (male=1, female=2), race distance (265 m, 420 m or 470 m) or adjusted rank finish position (rank) (1 to 6) and pre-race or post-race salivary log cortisol concentration (ng/ml) or heart rate (beats per minute, bpm) in British racing Greyhounds. Each association was found to be not significant ($p > 0.05$).

The distance travelled to the stadium by motor vehicle on the date of sample collection ranged from 1.61 km to 137.28 km, with a mean of 50.26 km and SD of 34.67 (SE 4.27). All Greyhounds were transported to the stadium in vans, with no further details available regarding the vehicle type or cage sizes within the vehicle. There were no associations between journey distance to the stadium and pre-race ($R^2 = 0.0368$) (Figure 6.22) or post-race ($R^2 = 0.0075$) (Figure 6.23) salivary log cortisol concentration. Additionally, there were no associations between journey distance to the stadium and pre-race ($R^2 = 0.00005$) (Figure 6.24) or post-race ($R^2 = 0.001$) (Figure 6.25) HR.

The racing age of the Greyhound ranged from 16 months to 84 months, with a mean of 36.98 months and SD of 12.48 (SE 1.58). No associations were identified between Greyhound racing age and pre-race ($R^2 = 0.0071$) (Figure 6.26) or post-race ($R^2 = 0.005$) (Figure 6.27) salivary log cortisol concentration, or between Greyhound racing age and pre-race ($R^2 = 0.005$) (Figure 6.28) or post-race ($R^2 = 0.006$) (Figure 6.29) HR.

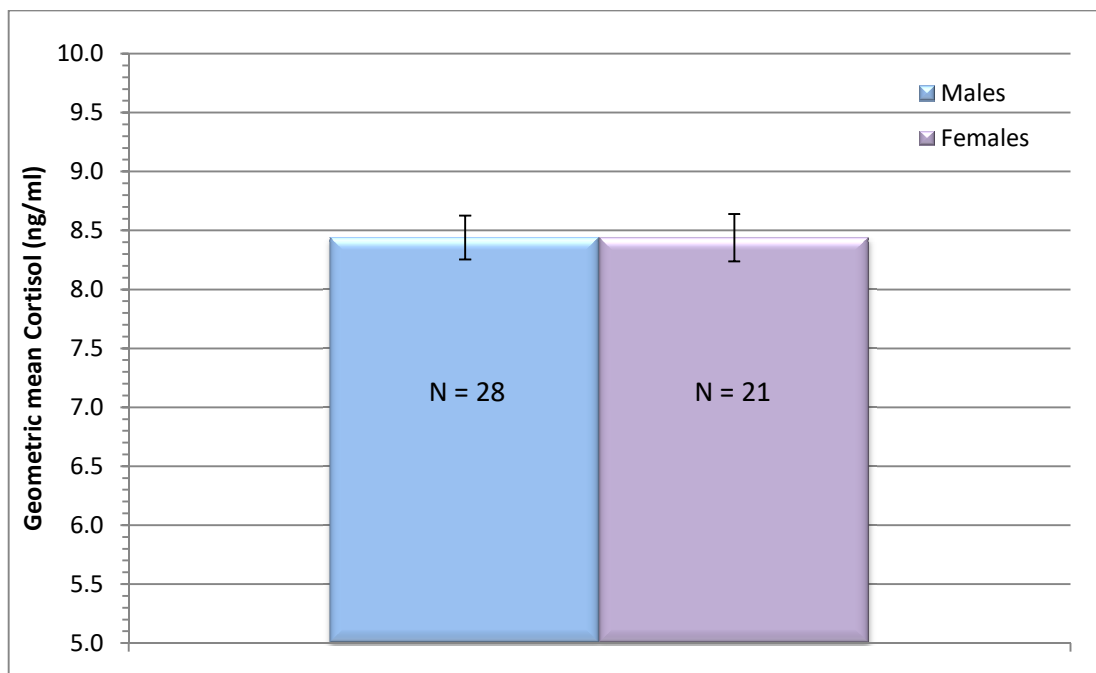


Figure 6.14. Pre-race geometric mean Greyhound salivary cortisol concentration (ng/ml) with respect to sex (male or female) (n = 49). Error bars indicate the standard error of the mean.

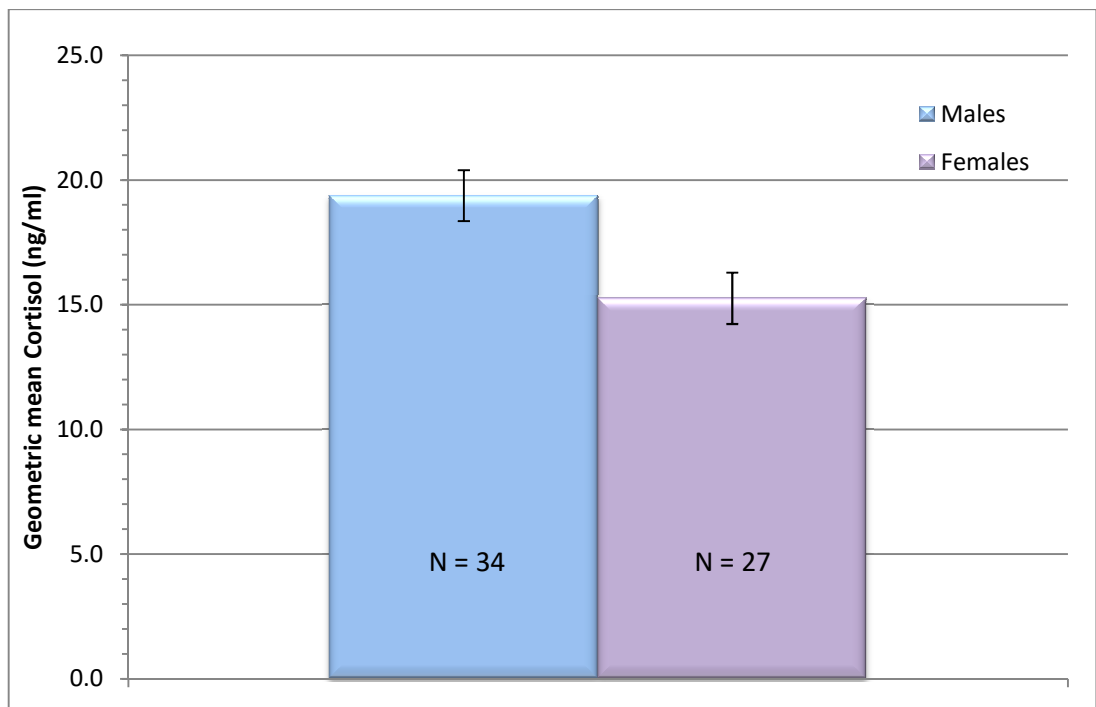


Figure 6.15. Post-race geometric mean Greyhound salivary cortisol concentration (ng/ml) with respect to sex (male or female) (n = 61). Error bars indicate the standard error of the mean.

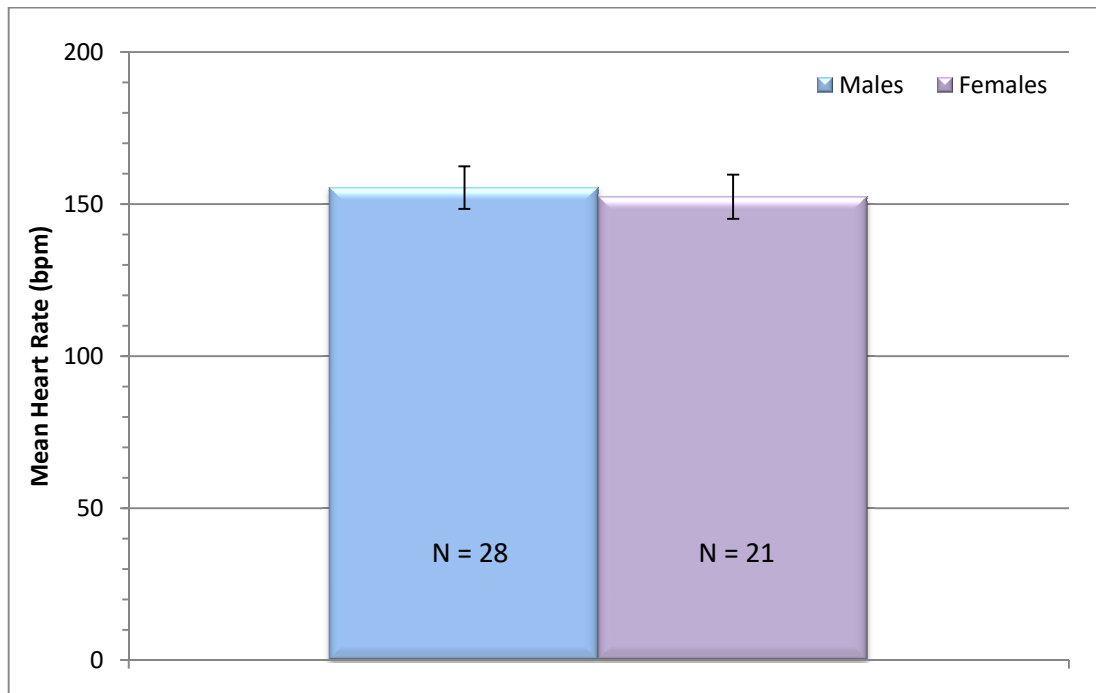


Figure 6.16. Pre-race mean Greyhound heart rate (beats per minute) with respect to sex (male or female) (n = 49). Error bars indicate the standard error of the mean.

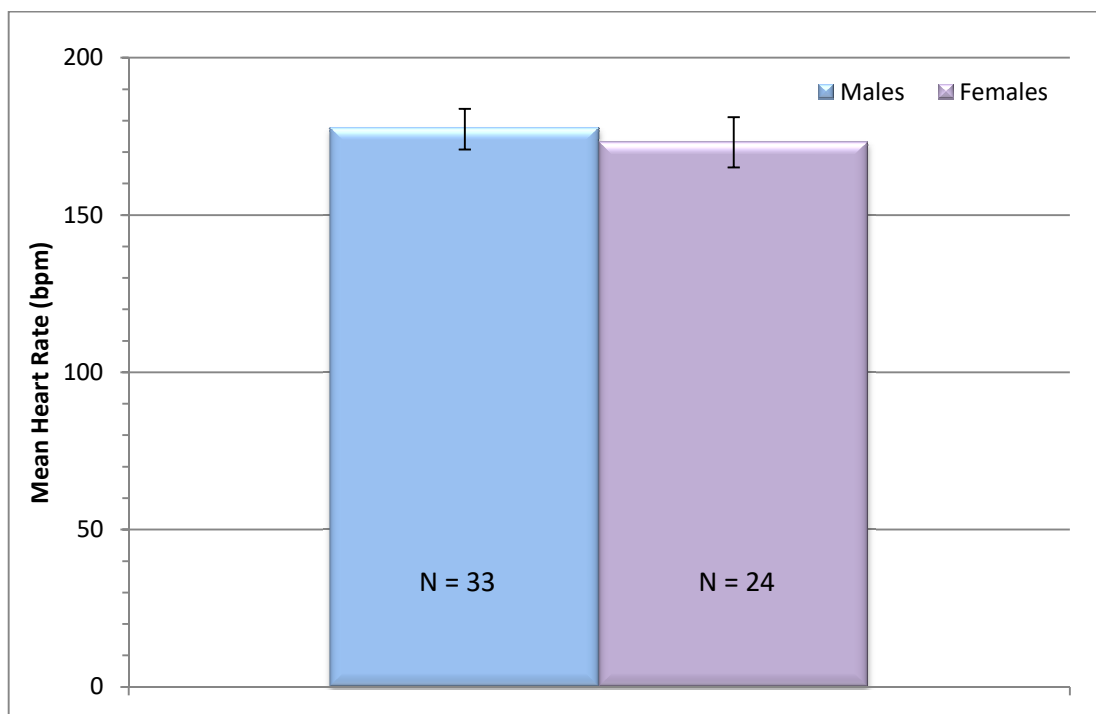


Figure 6.17. Post-race mean Greyhound heart rate (beats per minute) with respect to sex (male or female) (n = 57). Error bars indicate the standard error of the mean.

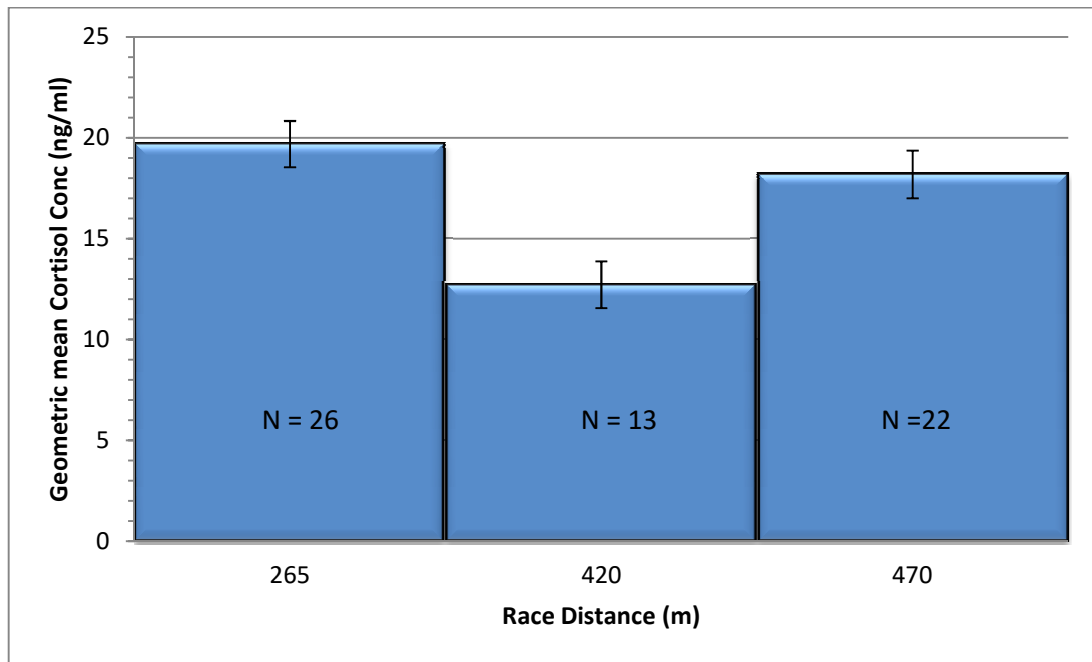


Figure 6.18. Post-race geometric mean Greyhound salivary cortisol concentration (ng/ml) with respect to race distance (metres) (n = 61). Error bars indicate the standard error of the mean.

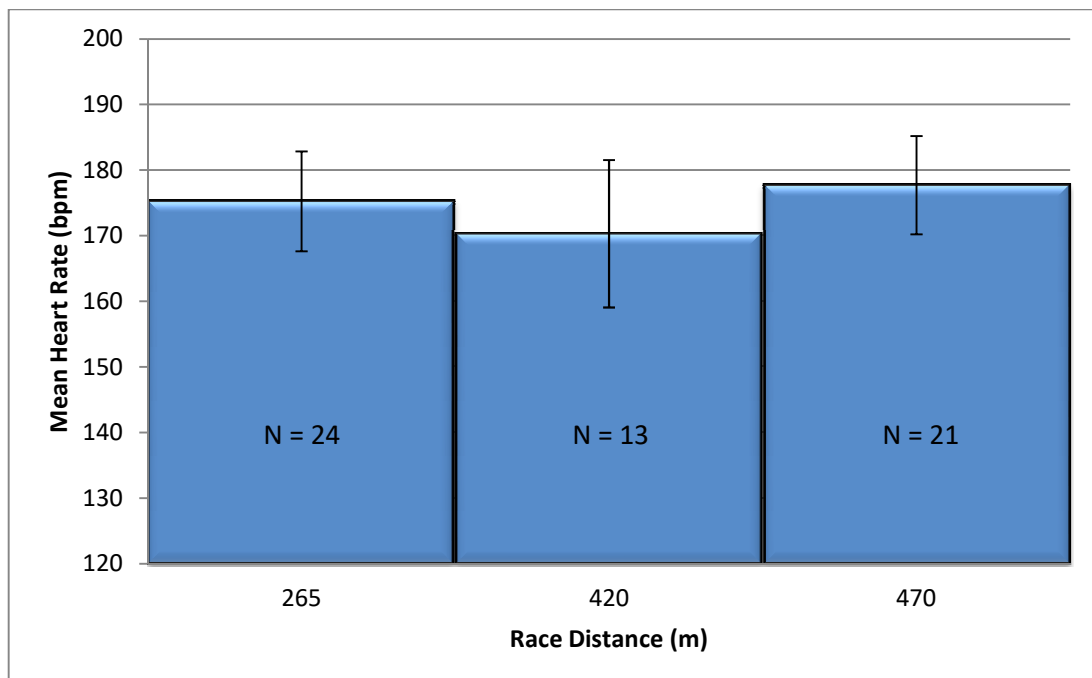


Figure 6.19. Post-race mean Greyhound heart rate (beats per minute) with respect to race distance (metres) (n = 58). Error bars indicate the standard error of the mean.

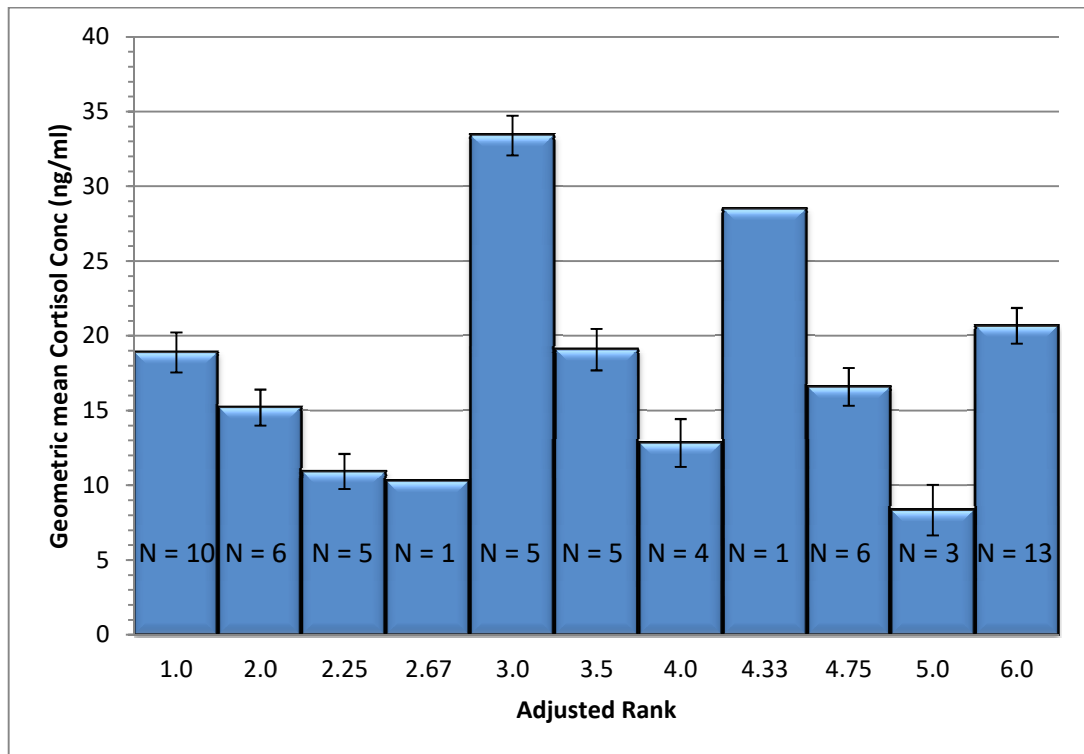


Figure 6.20. Post-race geometric mean salivary cortisol concentration (ng/ml) with respect to adjusted rank finish position (1-6) of the Greyhound (n = 59). Error bars indicate the standard error of the mean.

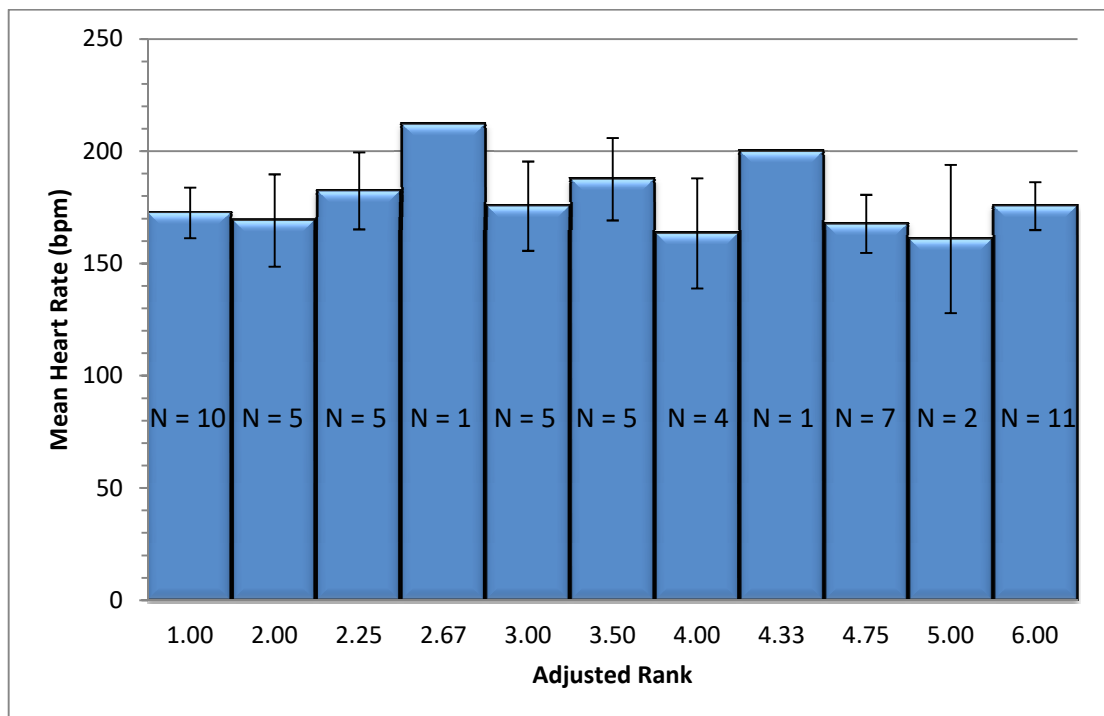


Figure 6.21. Post-race mean heart rate (beats per minute) with respect to adjusted rank finish position (1-6) of the Greyhound (n = 56). Error bars indicate the standard error of the mean.

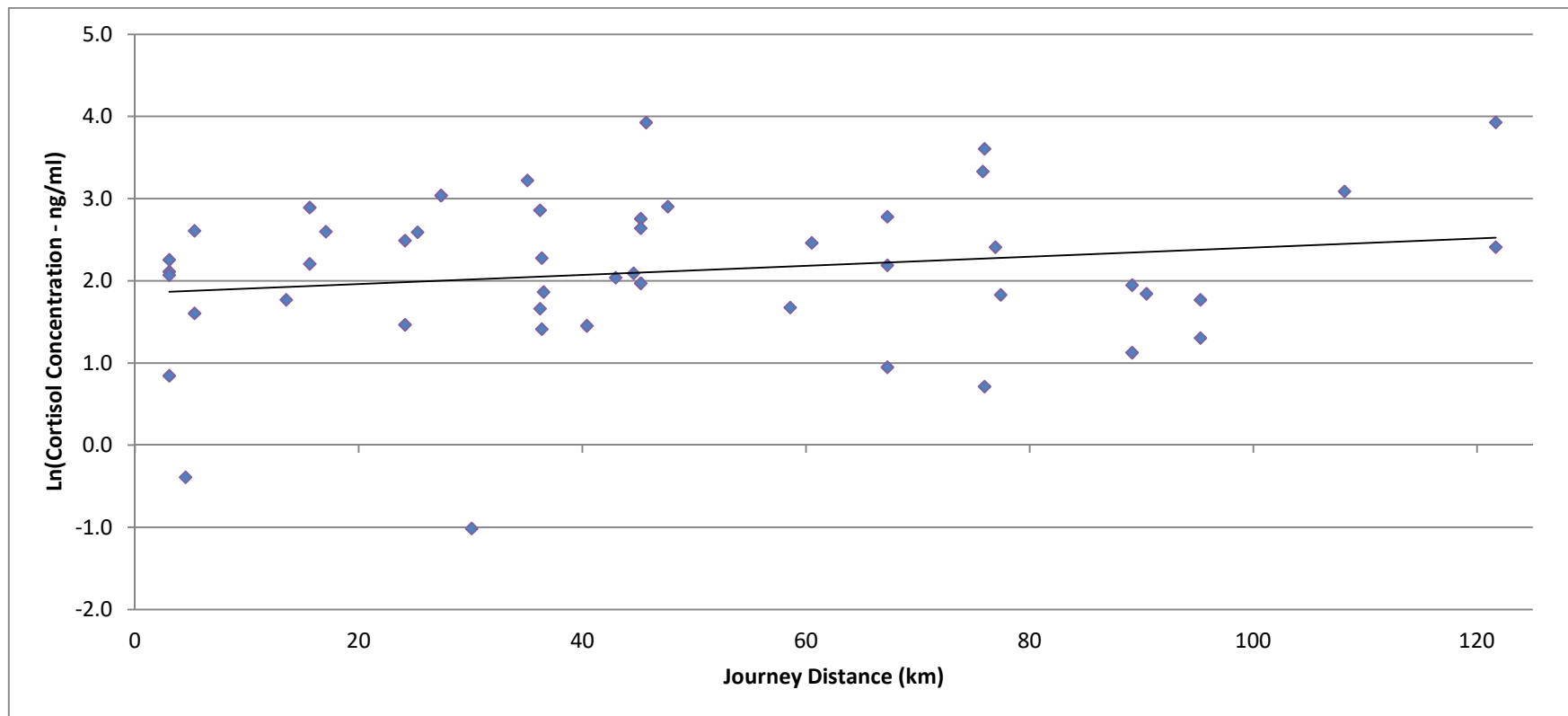


Figure 6.22. Pre-race Greyhound salivary log cortisol concentration (ng/ml) with respect to distance (kilometres) travelled by motor vehicle to the stadium ($n = 49$) ($R^2 = 0.0368$).

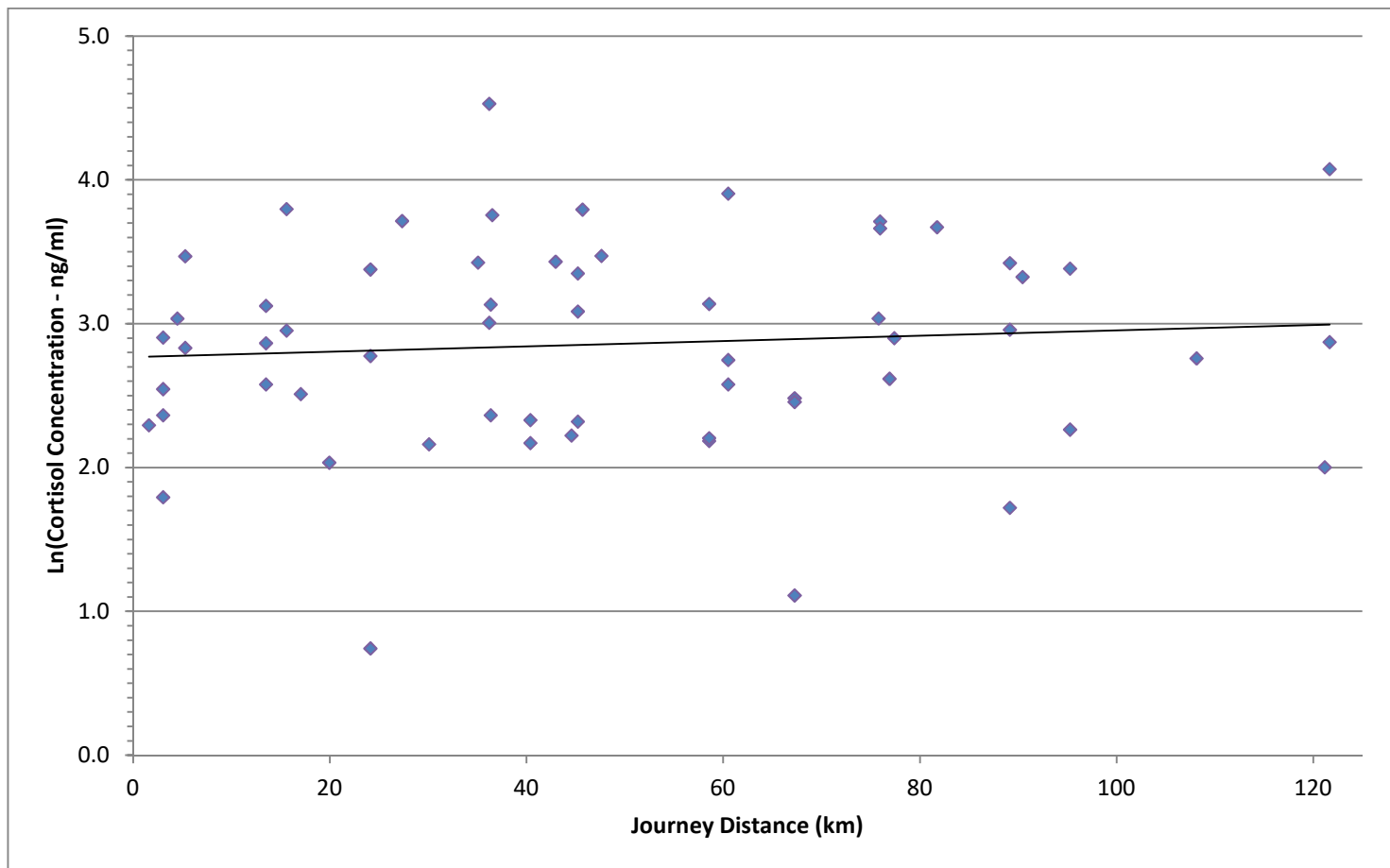


Figure 6.23. Post-race Greyhound salivary log cortisol concentration (ng/ml) with respect to distance (kilometres) travelled by motor vehicle to the stadium ($n = 62$) ($R^2 = 0.0075$).

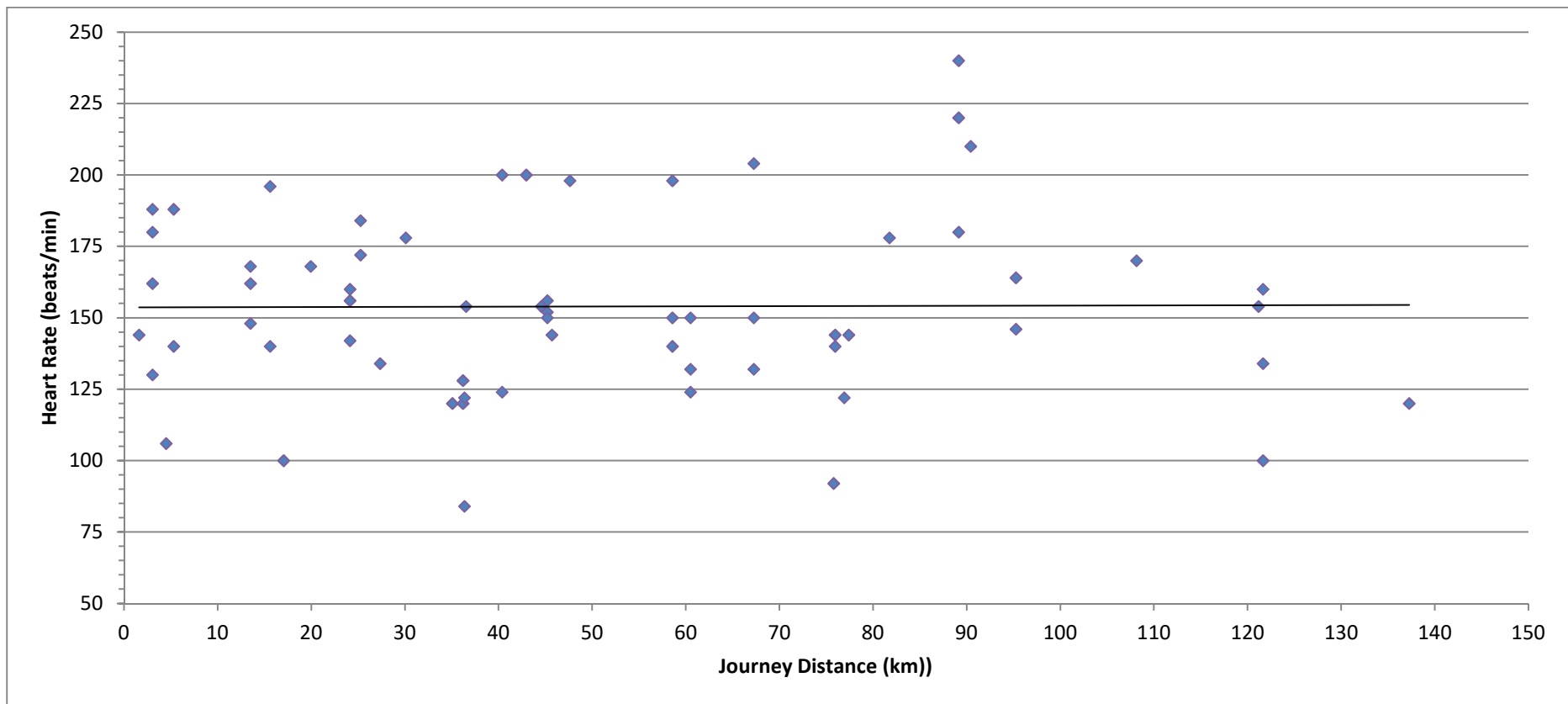


Figure 6.24. Pre-race Greyhound heart rate (bpm) with respect to distance (kilometres) travelled by motor vehicle to the stadium (n = 65) ($R^2 = 0.00005$).

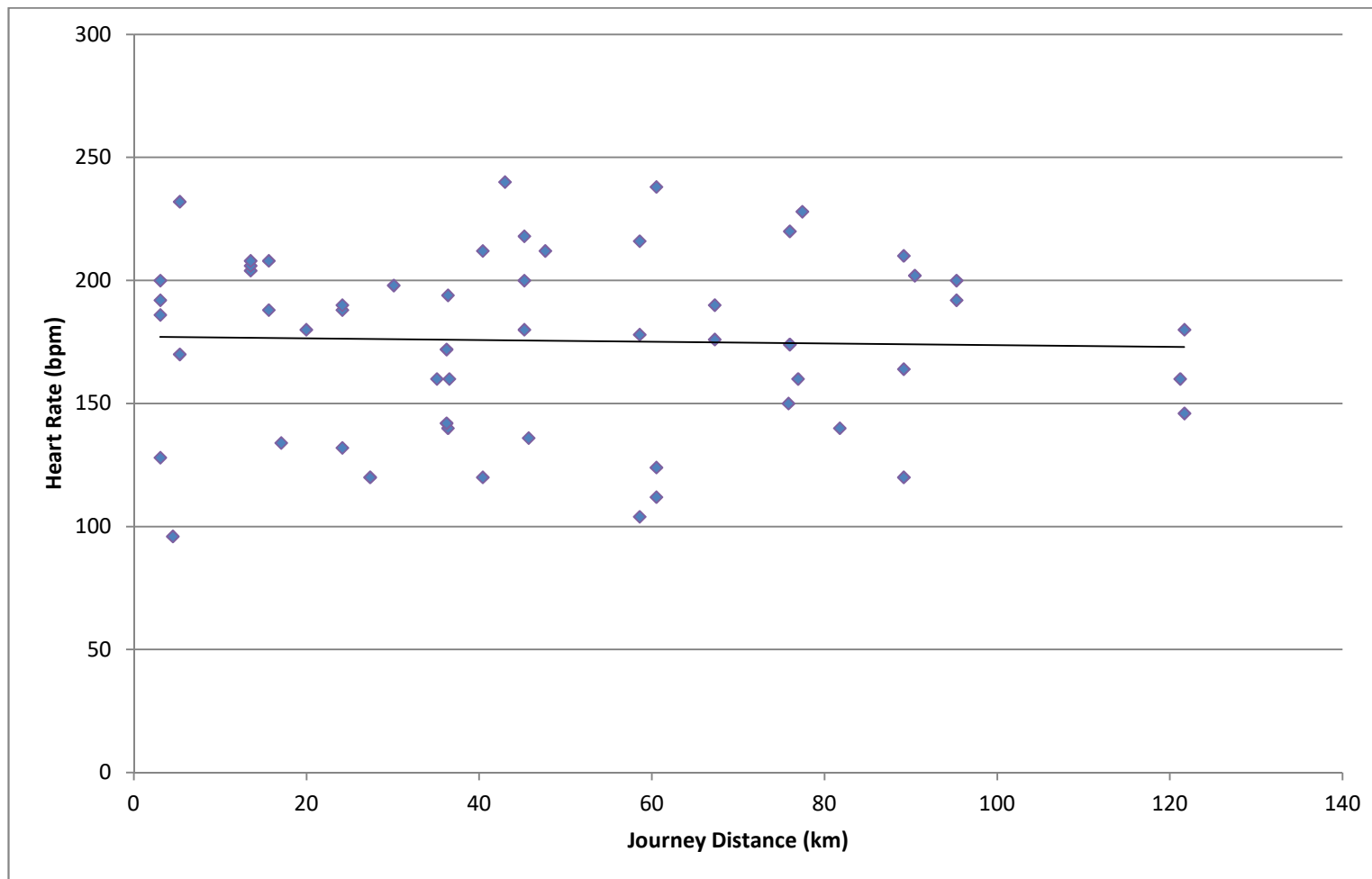


Figure 6.25. Post-race Greyhound heart rate (bpm) with respect to distance (kilometres) travelled by motor vehicle to the stadium (n = 58) ($R^2 = 0.001$).

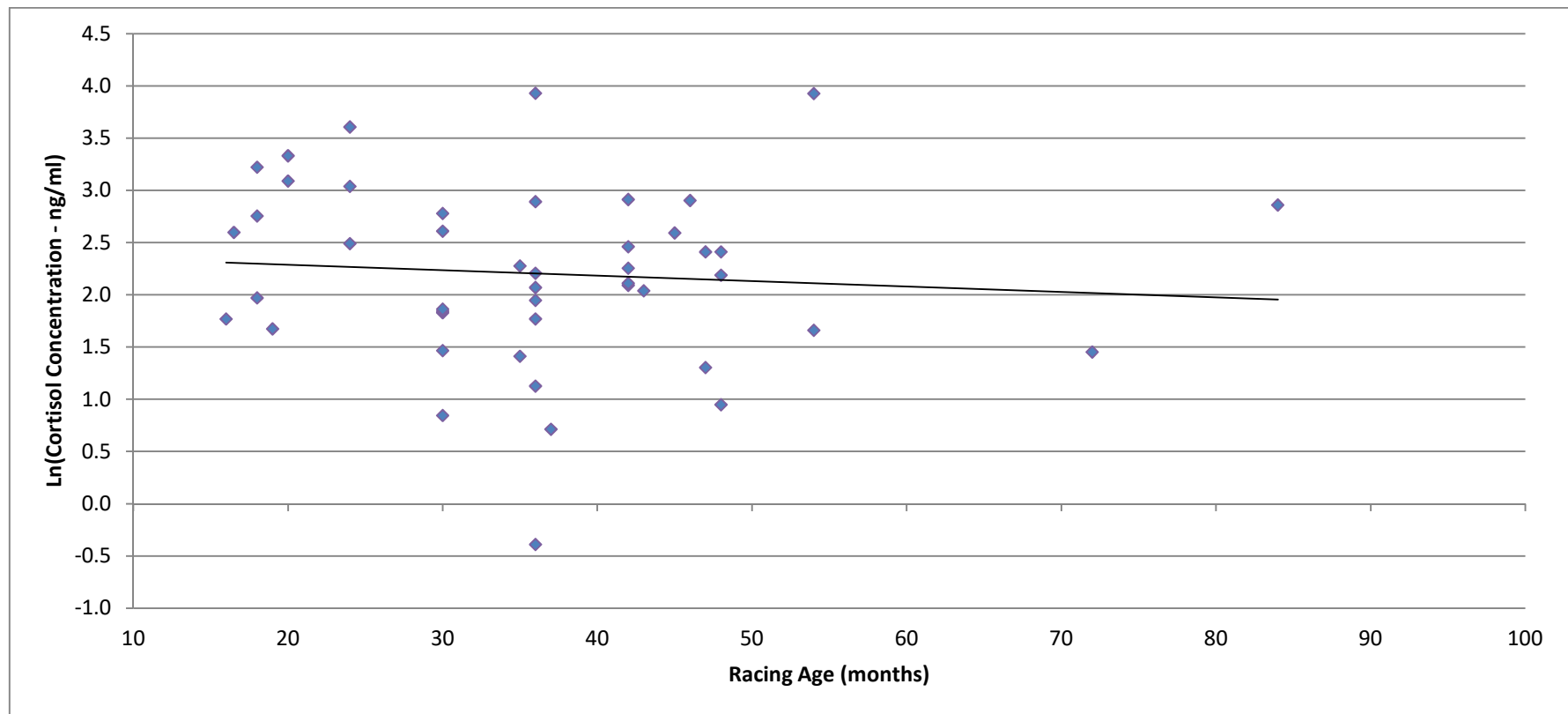


Figure 6.26. The mean pre-race salivary log cortisol concentration (ng/ml) with respect to racing age (months) of the Greyhound (n = 49) ($R^2 = 0.0071$).

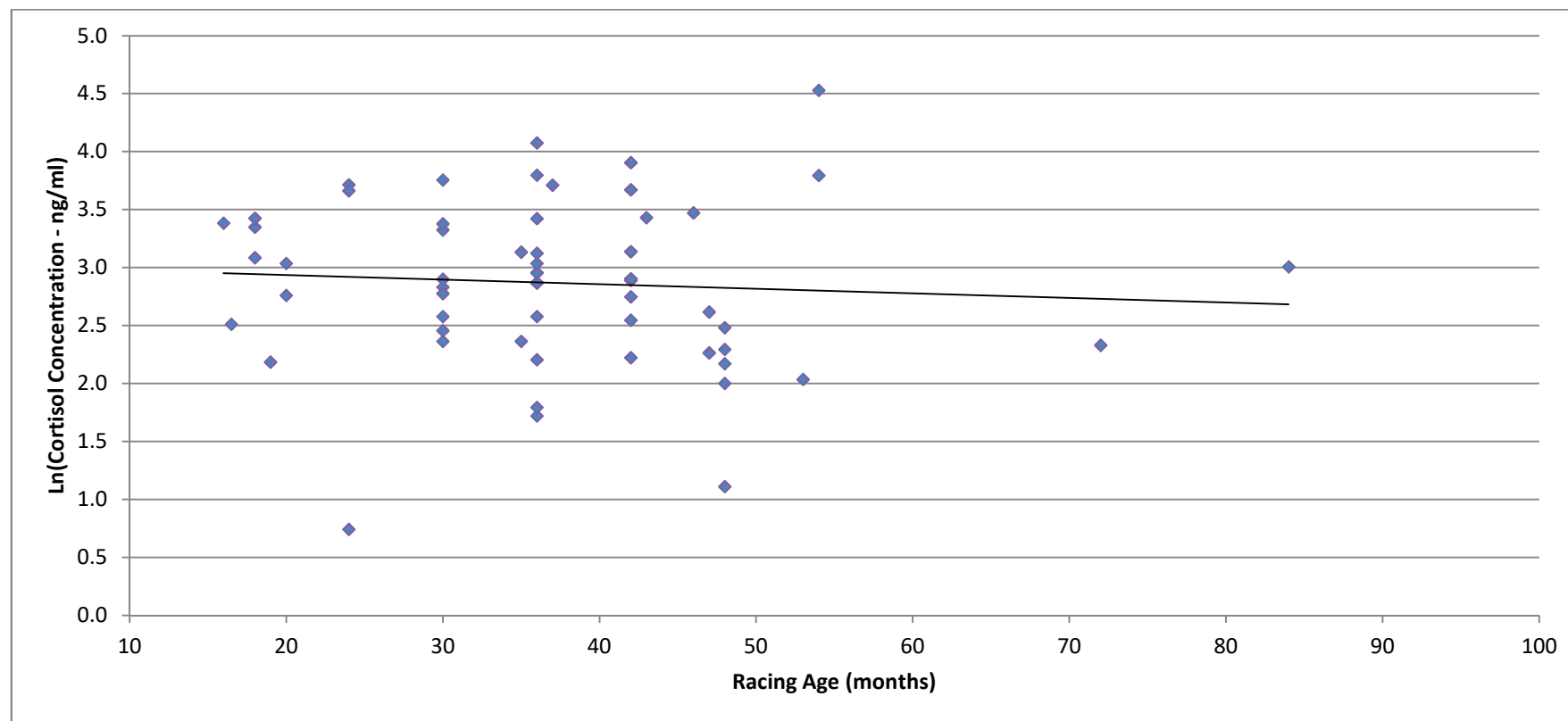


Figure 6.27. The mean post-race salivary log cortisol concentration (ng/ml) with respect to racing age (months) of the Greyhound ($n = 62$) ($R^2 = 0.005$).

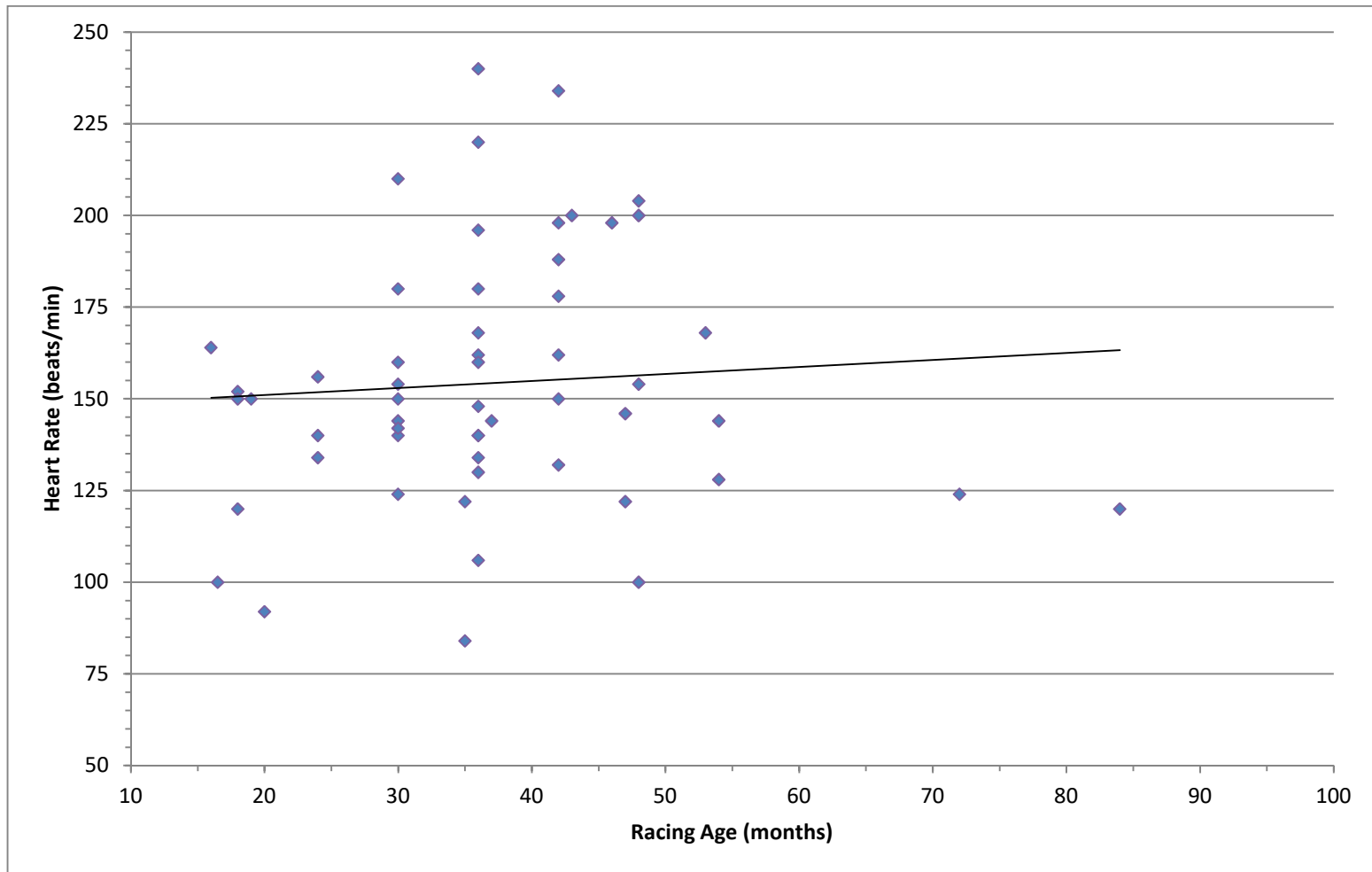


Figure 6.28. The mean pre-race heart rate (beats per minute) with respect to racing age (months) of the Greyhound ($n = 65$) ($R^2 = 0.005$).

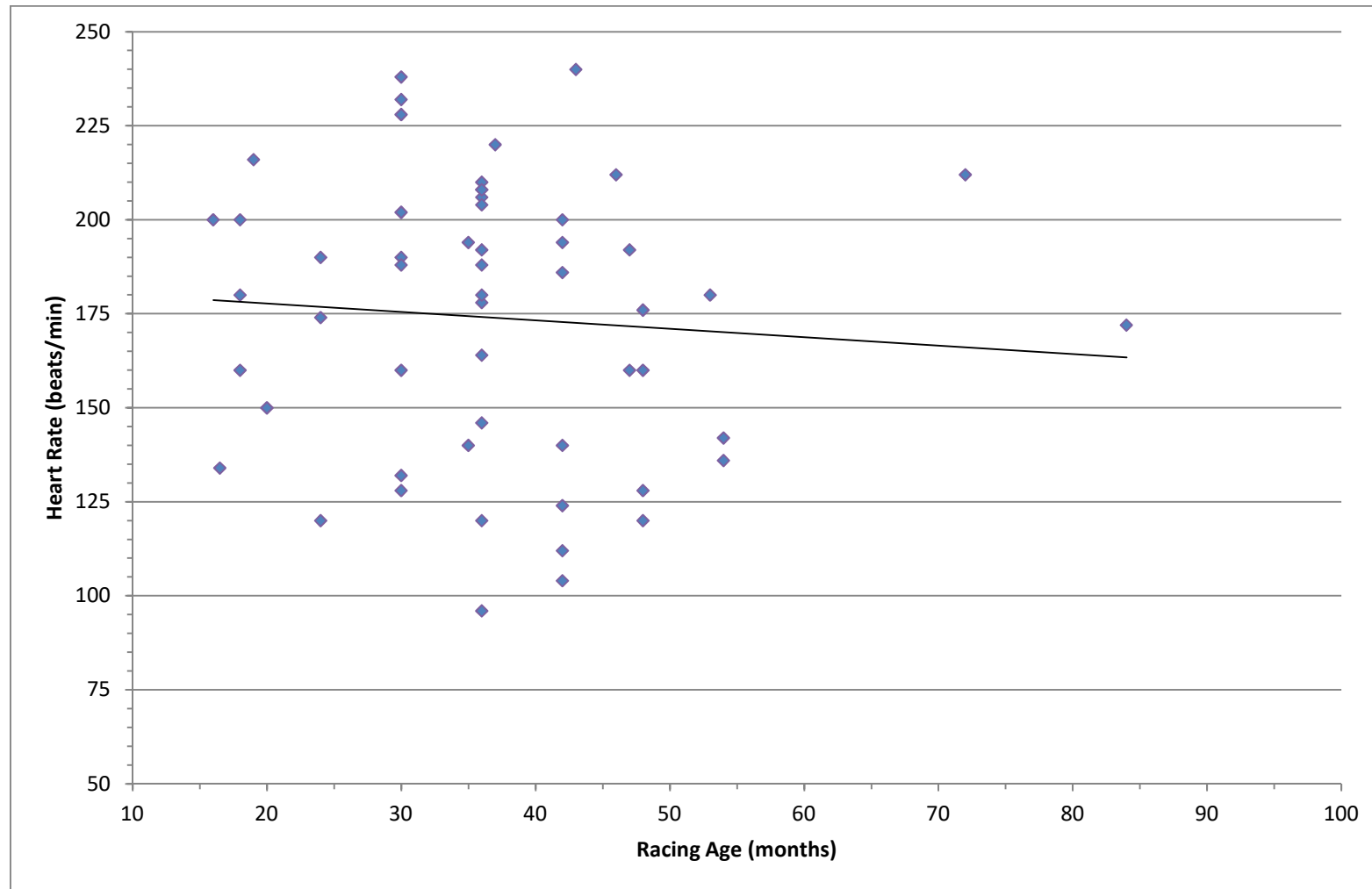


Figure 6.29. The mean post-race heart rate (beats per minute) with respect to racing age (months) of the Greyhound ($n = 58$) ($R^2 = 0.006$).

6.5. Discussion

The present study is the first to investigate measures of the stress response to short duration high-intensity exercise activity in British Greyhounds. Two measures of stress, salivary cortisol concentration and HR, were measured immediately pre- and post-racing for a total of 59 individuals. Additionally, the study considered factors of journey distance to the stadium, Greyhound sex, racing age, distance of the race and rank finish position on cortisol concentration and HR.

6.5.1. Effect of Short Duration, High-Intensity Exercise on Salivary Cortisol Concentration and Heart Rate in the Racing Greyhound

The pre-race and post-race salivary cortisol concentration ranged from 0.36 ng/ml to 50.89 ng/ml and from 2.10 ng/ml to 92.74 ng/ml, respectively. The mean pre-race and post-race salivary cortisol concentrations were 12.17 ng/ml (SD 10.95) and 21.71 ng/ml (SD 15.63), respectively. Basal salivary cortisol concentration was not measured in the present study, but has been reported to range between 0.8 ng/ml and 5.3 ng/ml in dogs (Glenk *et al.*, 2013; Stracke *et al.*, 2011; Vincent and Michell, 1992). The mean Greyhound cortisol concentration pre-race was therefore considerably higher than canine baseline values, indicating increased arousal and activation of the HPA axis during the immediate pre-race period.

The pre-race and post-race HR ranged from 84 bpm to 240 bpm and from 96 bpm to 240 bpm, respectively. The mean pre-race and post-race HR were 155.14 bpm (SD 32.90) and 175.03 bpm (SD 36.43), respectively. The conditioned racing Greyhound has a relatively low resting HR of 29 bpm to 48 bpm, increasing to 290 bpm to 420 bpm during maximal exercise (Reece *et al.*, 2015). In the present study, the mean pre-race HR was therefore considerably higher than resting values, indicating increased activity of the autonomic nervous system during the immediate pre-race period.

There were significant differences pre-race and post-race between each Greyhound's paired salivary log cortisol concentrations ($p = 0.000004$) and between each Greyhound's paired HR ($p = 0.002$). The post-race values were significantly higher than pre-race values for both salivary log cortisol concentration and HR, with mean differences of 0.78 ng/ml (SD 1.02) and 19.72 bpm (SD 46.29), respectively.

HR can vary according to physiological requirements. During exercise, the increased requirement for oxygen delivery to body tissues and excretion of carbon dioxide results in an elevation in HR, according to the intensity and duration of the activity. In the Greyhound, HR increases rapidly at the onset of exercise and reaches maximum levels within 30 s to 45 s (Reece *et al.*, 2015). In addition, exercise may cause an increase in cortisol levels, resulting in release of glucose and stores of fatty acids in an adaptive response. During exercise in humans, cortisol is released to assist in energy production once oxygen consumption above 50%

maximal oxygen consumption ($\text{VO}_2 \text{ max}$) has been attained (Buono *et al.*, 1986; Luger *et al.*, 1987). During a sprint race, Greyhounds are working at 100% $\text{VO}_2 \text{ max}$, however the short duration of exercise is primarily sustained through anaerobic metabolism of glucose and glycogen stores. Consequently, the racing Greyhound does not experience sufficient negative energy balance to facilitate cortisol release for fatty acid utilisation and protein catabolism (Wakshlag *et al.*, 2004).

The results obtained in the present study are in concordance with those reported in several previous studies investigating pre- and post-exercise cortisol concentrations in dogs. Pastore *et al.* (2011) measured salivary cortisol in several dog breeds immediately before and after a canine agility competition, and reported a significant ($p < 0.05$) increase in post-exercise cortisol concentration for all dogs. In addition, Durocher *et al.* (2007) reported a significant increase in urinary cortisol concentration following prolonged endurance exercise in Alaskan sled dogs, compared with values for unexercised control dogs. Rovira *et al.* (2007), however, found no significant difference between serum cortisol concentrations in dogs of various breeds immediately after an agility test, compared to basal values. In addition, Hinchcliff *et al.* (1993) reported that plasma cortisol concentration did not change significantly in sled dogs participating in a long-distance endurance race.

It has been suggested that the discrepancies in reported results may be partly due to adaptation of the HPA axis in response to repeated acute or chronic exposure to a particular stressor (Mastorakos *et al.*, 2005). Sustained physical activity in highly

trained human (Wittert *et al.*, 1996) and animal (Veissier and Boissy, 2007) athletes has been associated with a decreased HPA response to subsequent or continued exercise stress. Racing Greyhounds, however, are highly trained athletes subjected to a regular training and racing regime involving frequent high-intensity exercise activity. In the present study, the statistically significant ($p = 0.000004$) increase in post-race salivary cortisol concentration compared to pre-race values suggest that adaptation of the HPA axis may not have occurred in these dogs. In order to investigate the HPA response to repeated exercise stress in Greyhounds, both pre- and post-race cortisol levels of Greyhounds at various stages of their racing career, and at various training and racing intensities, could be examined in future work.

A limitation of the present study is that resting salivary cortisol concentrations and HR (on non-race days) were not measured. Consequently, the pre- and post-race cortisol levels and HR cannot be compared to basal values. Human athletes may experience pre-performance stress as a result of anticipation of the event (Heilbrun, 2004), and a similar emotional response has been reported in agility dogs prior to competition (Pastore *et al.*, 2004). A study of sled dogs by Angle *et al.* (2009) reported a significant increase in pre-exercise serum cortisol concentration compared to resting levels, due to anticipation of the event. There was a further increase in cortisol level following short duration high-intensity sprint exercise. Additionally, the anticipatory response to exercise is demonstrated by an increased resting HR in racing Greyhounds (Reece *et al.*, 2015). It is therefore likely that pre-race cortisol levels and HR were elevated in the present study compared

to basal resting values, with several contributing factors such as transport to the stadium, anticipation of the race event, spectator presence and pre-race veterinary examination. Further work is required to establish basal cortisol levels and HR for racing Greyhounds, by collecting samples and measurements from dogs at their residential kennels on non-race days. Further sampling and measurements before, during and after their journey to the race track may help to determine the true extent of fluctuations in cortisol concentration and HR attributed to transport, anticipation of the race event and subsequent high-intensity sprint exercise.

In the present study, all the Greyhounds taking part in a particular race were sampled simultaneously both pre- and post-race. The post-race samples were collected immediately after the Greyhound was walked off the racetrack and had returned to the paddock area, within five minutes of the end of the race. Consequently, there was a short delay in the collection of saliva samples and HR measurements post-race. HR starts to decrease rapidly within one or two minutes after the end of exercise (Reece *et al.*, 2015), as the Greyhound enters the post-exercise recovery phase. In a study of racing Greyhounds by Gillette *et al.* (2011), HR had returned to resting levels approximately 30 minutes after running. Consequently, the post-race HR measurements collected in the present study may not be a true indication of the immediate post-race values, and this is a limitation of the study design.

Several authors have reported a delay, however, in the increase in canine plasma or salivary cortisol in response to a stressor (Engeland and Gann, 1989; Vincent

and Michell, 1992). In a study of dogs by Engeland and Gann, (1989), the increase in plasma cortisol did not begin until after the stressor (three minutes of noise) had ended, and peaked approximately 11 minutes after the noise began. Due to the short duration of Greyhound races (usually less than two minutes) and the lag in increase in salivary cortisol, saliva samples collected immediately at the finish line may, in fact, be measuring stress experienced by the Greyhound during the pre-race period. Consequently, the short delay (maximum five minutes) in collection of post-race saliva samples in the present study may have resulted in salivary cortisol concentrations that more accurately measure the stress experienced by the Greyhound during the actual race. In dogs, a return to basal salivary cortisol levels has been reported to take approximately 30 minutes after removal of the stressor (Vincent and Michell, 1992).

In the present study, saliva samples and HR measurements were collected simultaneously for each Greyhound, both pre- and post-race. No correlations were found, however, between Greyhound salivary log cortisol concentration and HR in pre-race ($R^2 = 0.0098$) (Figure 6.12) or post-race ($R^2 = 0.0104$) (Figure 6.13) measurements. This result may be explained by the delay in the increase in plasma or salivary cortisol in response to a stressor (Engeland and Gann, 1989; Vincent and Michell, 1992), in comparison to the rapid variations in HR that may occur in response to changing internal or external stimuli. In addition, an animal's resting HR, their HR during maximal exercise, and how quickly their HR returns to resting levels post-exercise are all affected by the animal's physical fitness (Reece *et al.*, 2015). In future studies, it would be useful to investigate HR variability (short-term

fluctuations in HR) as a measure of the response of the autonomic nervous system to stress, using a continuous HR monitoring system (Becker-Birck *et al.*, 2013; Lewinski *et al.*, 2013; Schmidt *et al.*, 2010a,b,c).

6.5.2. Influence of Sex and Racing Age on Salivary Cortisol Concentration and Heart Rate in the Racing Greyhound

The sex of the Greyhound was found to have no significant association ($p > 0.05$) with pre- or post-race salivary log cortisol concentration, and no significant association ($p > 0.05$) with pre- or post-race HR. No associations were identified between Greyhound racing age and pre-race ($R^2 = 0.0071$) (Figure 6.26) or post-race ($R^2 = 0.005$) (Figure 6.27) salivary log cortisol concentration, or between Greyhound racing age and pre-race ($R^2 = 0.005$) (Figure 6.28) or post-race ($R^2 = 0.006$) (Figure 6.29) HR. These results are in agreement with the study of pre- and post-competition salivary cortisol levels in agility dogs by Pastore *et al.* (2011), in which age, body size and sex of the dog had no significant association with cortisol concentration. Reimers *et al.* (1990), however, reported that basal plasma cortisol levels are influenced by the age of the dog.

6.5.3. Influence of Race Distance on Salivary Cortisol Concentration and Heart Rate in the Racing Greyhound

The race distance (265 m to 470 m) was found to have no significant association ($p > 0.05$) with post-race salivary log cortisol level or HR in racing Greyhounds. In

contrast, Durocher *et al.* (2007) reported a significant ($p < 0.05$) increase in urinary cortisol concentrations with increasing race distance in sled dogs performing strenuous exercise. Racing Greyhounds typically take part in short-duration high-intensity sprint activity competing over race distances of 220 m to 1,080 m with maximum speeds of 18 m/s (Dobson *et al.*, 1988). Sled dogs, however, typically compete in prolonged endurance exercise. The differences in reported results may therefore reflect differences in exercise intensity and duration, as well as physiological variations between racing Greyhounds and sled dogs.

6.5.4. Influence of Rank Finish Position as a Measure of Race Performance on Salivary Cortisol Concentration and Heart Rate in Racing Greyhounds

The study was performed at an Independent (non-GBGB licensed) race track. As a result, measures of Greyhound race performance were limited to rank finish position in the race, and actual race times or speeds were unavailable. The rank finish position (one to six) had no significant association ($p > 0.05$) with pre- or post-race log cortisol level or HR, suggesting that the physiological and/or psychological stress experienced by the Greyhound before and during racing may be unrelated to their race performance. The rank finish position, however, reflects the performance of the Greyhound relative to that of the other dogs competing in the race. To further investigate and quantify the association of Greyhound race performance with cortisol levels and HR, it would be useful to consider the

performance measurements of race time over a specific race distance or mean racing speed.

6.5.5. Influence of Travel Distance on Salivary Cortisol Concentration and Heart Rate in the Racing Greyhound

On the dates of sample collection, Greyhounds had travelled between 1.61 km to 137.28 km by road in order to attend the race meeting. Based on owner and trainer responses, the reported duration of this journey ranged from five minutes to three hours. Consequently, there was considerable variation in the length of time the Greyhound had been travelling in a motor vehicle prior to the pre-race saliva sample collection and HR measurement. No associations were identified between journey distance to the stadium and pre-race ($R^2 = 0.0368$) (Figure 6.22) or post-race ($R^2 = 0.0075$) (Figure 6.23) salivary log cortisol concentration. Additionally, there were no associations between journey distance to the stadium and pre-race (Figure 6.24) or post-race (Figure 6.25) HR.

Travelling in a motor vehicle is a potentially stressful event for most animals. Stressful stimuli initiate adrenomedullary and sympathetic nervous system responses, resulting in release of epinephrine and an increase in HR. Studies have demonstrated that transport is stressful for horses, based on increased cortisol secretion, increased HR and changes in HR variability (Fazio *et al.*, 2008, 2013; Medica *et al.*, 2010; Schmidt *et al.*, 2010a,b,d). Bergeron *et al.* (2002) reported an increase in plasma cortisol levels of Beagles following road transport, compared to

basal values. Additionally, loading and unloading procedures resulted in the greatest increase in HR during air transport of the dogs (Bergeron *et al.* 2002).

In the present study, basal salivary cortisol concentration and HR were not measured. Following transport of the Greyhound to the stadium, the mean pre-race salivary cortisol concentration and HR were 12.17 ng/ml (SD 10.95) and 155.14 bpm (SD 32.90), respectively. These values are considerably higher than the reported basal salivary cortisol level in dogs (0.8 ng/ml to 5.3 ng/ml) (Glenk *et al.*, 2013; Stracke *et al.*, 2011; Vincent and Michell, 1992) and the resting HR of a conditioned racing Greyhound (29 bpm to 48 bpm) (Reece *et al.*, 2015). This suggests that transport to the stadium may have contributed to increased stress experienced by the Greyhound during the pre-race period, however the level of stress is not associated with journey distance. These results are in agreement with a study of cortisol levels in stallions before and after road transport over different distances (100 km, 200 km and 300 km), in which persistent increases in serum cortisol showed no differences between the various distances and durations of travel (Fazio *et al.*, 2008). Schmidt *et al.* (2010a) reported that cortisol release is stimulated early on during transport of horses, and stimulation of cortisol release occurs almost as effectively for short-duration transport as for transport over longer distances.

Given the high frequency of travel for most racing Greyhounds, these results warrant further investigation of measures of transport-related stress. In future work, salivary cortisol and HR measurements collected before, during and

immediately after transport to the stadium would be useful in order to measure the changes in stress during the journey.

6.5.6. Conclusions

In conclusion, the present study is the first to investigate the effects of exercise and various environmental factors on endocrine and HR indicators of stress in the racing Greyhound. The study has identified that high-intensity sprint exercise in Greyhounds results in an increased HPA response and subsequent increase in the release of salivary cortisol, which is detectable immediately post-racing. There were significant differences pre-race and post-race between each Greyhound's paired salivary log cortisol concentration ($p = 0.000004$) and paired HR ($p = 0.002$), with mean differences of 0.78 ng/ml (SD 1.02) and 19.72 bpm (SD 46.29), respectively. For both salivary cortisol concentration and HR, the post-race values were significantly higher than pre-race values. No correlations were found, however, between Greyhound salivary log cortisol concentration and HR in pre-race ($R^2 = 0.0098$) or post-race ($R^2 = 0.0104$) measurements. Greyhound sex, age, journey distance to the stadium, race distance and rank finish position in the race were not associated ($p > 0.05$) with pre- or post-race salivary log cortisol concentration or HR.

Further work is required to establish basal cortisol concentration and HR in racing Greyhounds on non-race days, as well as measurements before, during and after transport to the stadium on race days, in order to investigate the variation in

cortisol levels and HR due to transport stress, anticipation of the race event and subsequent high-intensity sprint exercise. Alternative measures of stress could also be considered, such as monitoring of blood pressure, gastrointestinal activity, immunological changes and behavioural responses (Bergeron *et al.*, 2002; Hekman *et al.*, 2012; Kay and Hall, 2009; Menor-Campos *et al.*, 2011; Moberg, 2000; Ottenheimer Carrier *et al.*, 2013; Pastore *et al.*, 2011). In future work, a larger sample size would be beneficial to improve statistical power and ability to detect any small but biologically significant variations between groups.

The study detailed in Chapter Three identified a number of environmental (non-genetic) factors that were significantly associated with the racing performance of British Greyhounds, including sex of the dog, birth year, racing age, number of dogs in the race, starting trap number, stadium, month-year-stadium combination of the race, race type and race distance. Future studies could investigate the influence of Greyhound arousal and HPA response on measures of racing performance such as race time and speed.

Frequent or chronic activation of the HPA axis through exposure to stressful stimuli is associated with adverse effects on animal growth rate, disease susceptibility, reproductive performance and behaviour (Johnson *et al.*, 1992). Bone physiology is known to be affected by glucocorticoid hormones such as cortisol, and high circulating levels of glucocorticoids are associated with an increased risk of osteoporosis and bone fracture in humans (Hardy and Cooper, 2010). The present study has identified that both pre-race and post-race mean salivary cortisol

concentrations in Greyhounds are considerably higher than reported basal values in dogs (Glenk *et al.*, 2013; Stracke *et al.*, 2011; Vincent and Michell, 1992). Greyhounds take part in frequent high-intensity exercise during training and racing throughout their careers. As a result, they are potentially exposed to repeated physiological stress with subsequent HPA axis activation and elevated cortisol secretion. Consequently, this may affect bone physiology, BMD and risk of fracture injuries.

Stress fracture is a common injury of the racing Greyhound (Bellenger *et al.*, 1981; Bergh *et al.*, 2012; Boudrieau *et al.*, 1984a, 1984b; Devas, 1961; Emmerson *et al.*, 2000; Gannon, 1972; Johnson *et al.*, 2000, 2001; Lipscomb *et al.*, 2001; Muir *et al.*, 1999; Tomlin *et al.*, 2000; Wendelburg *et al.*, 1988). It is hypothesised that stress fracture is a multifactorial multigenic disorder resulting from both genetic and environmental factors (Yanovich *et al.*, 2012). The study detailed in Chapter Five identified several nominally associated SNPs and regions of interest on chromosomes that may be implicated in the pathogenesis of stress fracture in the racing Greyhound. Future studies could investigate the influence of cortisol levels on the development of stress fracture injuries in the Greyhound, and whether elevations in cortisol as a result of stress experienced by the Greyhound during repeated training and racing affects their risk of injury.

In addition, there is considerable variation within animal populations in the extent of an individual's stress response to identical stressful stimuli (Pottinger, 2000). This suggests that the response of an animal to stress is affected by genetic as well as

environmental factors. Consequently, selective breeding may be used as a strategy for the reduction in responsiveness to stressors (Pottinger, 2000), in order to improve animal welfare. The extent of the stress response and ability of a Greyhound to cope with stressful stimuli such as transport, kennelling, veterinary examinations, spectator presence and racing are therefore important factors to consider in the selection of Greyhounds for breeding.

Chapter Seven.

General Discussion and Conclusions

7. General Discussion and Conclusions

7.1. Introduction

Greyhounds are elite athletes, capable of accelerating to speeds of up to 18 m/s in just a few seconds (Dobson *et al.*, 1988). Greyhound racing is the third largest spectator sport in the UK, and a popular off-course betting medium (Greyhound Board of Great Britain, 2009). A recent inquiry into the welfare of racing Greyhounds in England (APGAW, 2007) and an independent review of the British Greyhound industry (Donoughue, 2007), however, both identified a number of serious welfare concerns within the industry. In particular, the reports highlighted that the industry produces a surplus of approximately 6,000 to 12,000 unwanted young Greyhounds each year, that do not make the grade to race (APGAW, 2007).

The selection of Greyhounds for breeding is currently based on phenotypic racing performance; a slow and inefficient method of animal improvement (Taubert *et al.*, 2007). The aim of animal breeding is to accurately identify animals with ‘superior’ characteristics, and to select these individuals for breeding. Scientific methods of selection based on performance measurements, genetic evaluations and EBVs have been extremely successful in improving health, production, performance and trainability in many groups of animals (Bourdon, 2014; Famula, 2012; Simm, 1998). In contrast, there have been no previous genetic studies investigating the performance of British racing Greyhounds, and EBVs have not previously been calculated.

In addition, racing Greyhounds sustain several specific musculoskeletal injuries that are infrequent in other working or companion dogs (Davis, 1967; Hickman, 1975; Prole, 1976; Vaughan, 1969). Bone fracture injuries of the distal limb are common (Gannon, 1972; Prole, 1976; Sicard *et al.*, 1999). The majority are considered stress fractures, due to cyclic compressive loading causing an accumulation of micro-damage in the bone beyond its rate of repair by remodelling. Catastrophic fracture occurs through micro-crack dissemination or coalescence (Devas, 1961; Gannon, 1972; McBryde, 1975; Taylor 1997, 1998). Stress fracture is considered a multifactorial multigenic disorder resulting from the combined effects of both genetic and environmental factors (Yanovich *et al.*, 2012). There is indirect evidence suggesting the involvement of genetic factors in the pathogenesis of stress fractures in humans (Friedl *et al.*, 1992; Giladi *et al.*, 1986; Givon *et al.*, 2000; Linenger and Shwayhat, 1992; Milgrom *et al.*, 1985; Singer *et al.*, 1990; Van Meensel and Peers, 2010), and several candidate gene association studies have identified potentially associated polymorphisms (Chatzipapas *et al.*, 2009; Korvala *et al.*, 2010; McClung and Karl, 2010; Valimaki *et al.*, 2005b; Yanovich *et al.*, 2011, 2012). Investigation of genetic factors affecting the risk of stress fracture injuries in the racing Greyhound, however, has not previously been reported.

Cortisol is a glucocorticoid hormone secreted as a result of activation of the HPA axis in response to stressors (Matteri *et al.*, 2000; Mostl and Palme, 2002), and is frequently used as a measure of canine stress (Beerda *et al.*, 1996, 1998; Hekman *et al.*, 2012; Kobelt *et al.*, 2003; Pastore *et al.*, 2011). Previous studies have

investigated the effect of exercise on cortisol levels in agility and sled dogs (Angle *et al.*, 2009; Pastore *et al.*, 2011; Rovira *et al.*, 2007; Wakshlag *et al.*, 2004). The effects of exercise and racing on cortisol concentration in Greyhounds, however, have not previously been reported.

7.2. Aims

The aims of the thesis were to establish a performance and pedigree database for British racing Greyhounds in order to investigate a number of environmental (non-genetic) influences on Greyhound race performance; to perform the first genetic investigation of British Greyhound race performance data and estimate variance components for important performance traits within the industry; and to calculate EBVs for British Greyhounds enabling them to be ranked in order of their additive genetic merit. Additionally, the thesis aimed to establish a DNA archive for British racing Greyhounds; to perform a discovery GWA-study investigating genetic factors that may predispose racing Greyhounds to developing stress fracture injuries; and to investigate cortisol and HR indicators of Greyhound stress, pre- and post-racing.

7.3. Overall Findings and Conclusions

7.3.1. Investigation of Environmental Factors Affecting the Race Performance of British Greyhounds

A British Greyhound performance and pedigree database was created, consisting of 1,711,489 individual race performance records for 50,452 GBGB-licensed Greyhounds during a five-year period from 2008 to 2012, with a total of 73,344 Greyhounds in the pedigree file. In the largest study of its kind, the data set was examined using ANOVA statistical procedures and general linear models to investigate various environmental (non-genetic) influences on the race performance of British Greyhounds. The environmental factors of Greyhound sex, birth year, number of dogs in the race, starting trap number, stadium, month-year-stadium combination of the race, and the covariate racing age of the dog, were each found to be significantly associated ($p < 0.0001$) with the performance traits Race Time (over a 480 m race distance) and Speed (over all race distances). In addition, the factors of race type and the covariate race distance were each found to be significantly associated ($p < 0.0001$) with Speed. This result is to be expected, with decreasing mean speed as the race distance and number of bends to negotiate increases. The environmental factors of Greyhound sex, birth year, starting trap number and month-year-stadium combination of the race were each found to be significantly associated ($p < 0.0001$) with the performance trait Rank. The covariate racing age of the dog was also significantly associated ($p < 0.0005$) with Rank. Factors of race type, stadium, and the covariate race distance,

however, had no significant effect ($p > 0.05$) on Rank. This result is to be expected, as overall there would be an equal number of (adjusted) rank finish positions one to six regardless of race distance, number of bends to negotiate or stadium.

Male Greyhounds performed better than females in terms of Race Time (over a 480 m race distance), Speed and Rank (over all race distances). This finding would not necessarily result in a preference for male Greyhounds over females within the industry, as races are mixed sex and graded so that dogs compete against others of similar ability. One possible exception to this is for Open Races, in which any Greyhound of any ability may take part, and consequently a male dog may be considered more favourable.

British Greyhounds improved in Race Time and Speed until reaching maximum performance levels at approximately 30 months of age and 25 months of age, respectively, then declined in performance over the remainder of their racing career. These results would not necessarily impact the duration of a Greyhound's racing career as it is common for Greyhounds to drop down in race grades as they decline in speed with age, in order to continue racing competitively. Again, an exception to this may be for Open Race dogs, and this finding may demonstrate the optimum age for a Greyhound to take part in open races where speed and maximum performance are critical. Overall, Greyhounds born later in terms of birth year (2001 to 2011) tended to perform better, suggesting genetic improvement in the traits over time.

The number of dogs (4 - 6) in the race was significantly associated with Race Time (over a 480 m race distance) and Speed (over all race distances). Greyhounds performed better when there were five dogs competing in the race, and performed worst when there were four dogs. Six-dog races are most common in the UK, and four or five-dog races are typically held only when a Greyhound has been withdrawn from a race, for example due to injury, or where there are insufficient Greyhounds of similar ability available to take part in the race. In further work, it would be interesting to investigate whether the number of dogs taking part in the race is associated with injury rate. It may be expected that five-dog races result in fewer injuries compared to six-dog races, as there is less congestion of dogs. This may not be the case, however, as Greyhounds running in five-dog races were found to have faster mean speeds than those competing in six-dog races. Greyhound injury rate is reported to be higher with faster running speed (Sicard *et al.*, 1999).

Greyhounds performed better in terms of Race Time (over a 480 m race distance), Speed and Rank (over all race distances) when starting the race from trap number one, closest to the inner rail of the track, and performed worst when starting from trap five. This result is interesting, as for the vast majority of races the Greyhound's starting trap number is not randomly selected, but instead determined by the racing manager at each stadium. This finding may impact the British Greyhound industry, as trainers and owners of Greyhounds may feel disadvantaged if allocated trap number five. This would particularly apply to middle-running Greyhounds that tend to be allocated starting traps two to five,

rather than clear rail (trap one) or wide (trap six) runners. Furthermore, this finding may affect the betting industry considerably, by influencing the gambler's choice of Greyhound to place a bet on. Dogs starting from trap one won significantly more races over the study period, whereas dogs starting from trap five were least likely to win.

The stadium of the race was found to be significantly associated with Race Time (over a 480 m race distance) and Speed (over all race distances). This result may be explained by British Greyhound stadiums varying considerably in track design, track surface conditions and preparation methods. Due to the large data set and computational limitations of the software, it was not possible to examine the effect of local environmental conditions on Greyhound race performance in terms of variations between individual race events, or combinations of stadium and race date. The influence of season and climatic conditions was therefore examined through analysis of the effect of month-year-stadium combination of the race (MYStad). Climatic conditions can vary considerably, however, between days and hours in any given month in a particular location. It would be useful in future work to obtain local rainfall and temperature data for each stadium throughout the year, to further investigate the effects of season and climatic conditions on racing performance. Furthermore, the track surface conditions can vary considerably between races on a particular race night. Future work may involve analysis of a smaller subset of the data in order to investigate the influence of individual race event on Greyhound performance.

A further limitation of the study design is that it assumed each race start is independent. There is, however, clustering of the data, as the same dog can appear more than once in the performance results for each race start it completes during the study period.

Overall, there was a phenotypic improvement in British Greyhounds of 0.24 s in Race Time and 0.09 m/s in Speed, during the five-year period from 2008 to 2012. This is equivalent to a phenotypic improvement of 0.048 seconds per year and 0.018 metres per second per year for Race Time and Speed, respectively. Phenotypic improvements in Race Time and Speed may be observed as a result of both genetic improvement within the population and improvements in environmental (non-genetic) factors such as nutrition, training, climatic conditions and track surface conditions over time. It is not possible to achieve a phenotypic improvement in Rank over time, as a rank of one (first) is always the highest.

Additional environmental (non-genetic) influences on Greyhound race performance could be examined in future work, such as the effect of trainer, nutrition, Greyhound body weight and track surface water content. Additional measures of performance could also be examined, such as prize money earnings, total number of race starts and susceptibility to injury.

7.3.2. Genetic Analysis of Race Performance in British Greyhounds

This thesis presents the first study to investigate genetic variation in race results and estimate variance components and EBVs for performance traits of British Greyhounds. To date, it is the largest genetic study of racing Greyhound performance worldwide. The identified environmental factors were used to fit three univariate mixed linear animal-models, in order to perform genetic evaluations for each of the three performance traits: Race Time (over a 480 m distance), Speed and Rank (over all distances). Genetic parameters and EBVs were calculated using the software package DMU, version 6, release 5.2 (Madsen and Jensen, 2013), with estimation of variance components, fixed effects and random effects using AI-REML, BLUE and BLUP, respectively. Estimated heritabilities were found to be moderate-high for Race Time (0.44), moderate for Speed (0.37) and low for Rank (0.02). Repeatabilities were moderate-high for Race Time (0.56) and Speed (0.52), and low for rank (0.03). EBVs were calculated for 73,344 British Greyhounds, and varied from 3.340 (worst) to -1.090 (best) (SD 0.196) for Race Time, -0.786 (worst) to 0.666 (best) (SD 0.126) for Speed and 0.491 (worst) to -0.633 (best) (SD 0.098) for Rank. Over a 12 year period, genetic improvements equivalent to 0.021 s per year, 0.013 m/s per year and 0.0025 rank finish positions per year were identified for the performance traits Race Time, Speed and Rank, respectively.

The results indicate that the current method of Greyhound selection for breeding based on phenotypic performance is effective for Race Time and Speed, as both

traits are moderately heritable, and both phenotypic and genetic improvements in Race Time and Speed were observed over the five-year study period. The results also demonstrate, however, that the current system of breeding does not use the genetic potential of the population to maximum effect. Considerable variation in EBVs for Race Time (-0.031 to -0.426) (SD 0.09), Speed (0.032 to 0.240) (SD 0.05) and Rank (0.104 to -0.143) (SD 0.07) was identified in the top 20 Greyhound sires with the greatest numbers of offspring (817 to 3,203 progeny). The popular Greyhound sires used most frequently for breeding were not those with the best genetic merit.

The current over-breeding and production of surplus young Greyhounds is a significant welfare concern within the Greyhound industry. It is likely that a major factor in this overproduction and wastage is the inefficient selection of Greyhounds for breeding, with young dogs subsequently not performing as expected or as required. It is vitally important to select parents for breeding based on their genetic merit, rather than those that perform well simply due to favourable environmental conditions, as it is only the genetic superiority that will be passed on to the next generation. Whilst there is no need to increase the overall speed of the British Greyhound population, there is an urgent requirement to select Greyhounds for breeding more efficiently, so that dogs are not discarded at a young age when they are found to be too slow for competitive racing. The application of targeted genetic selection based on EBVs would be considerably more efficient than traditional methods of breeding based on phenotypic performance (Bourdon, 2014; Simm, 1998; Taubert *et al.*, 2007). Importantly, selection based on EBVs would result in

offspring of predictable and superior genetic merit for racing performance, therefore minimising the number of young Greyhounds that do not make the grade to race.

This thesis uses speed (and race time as a function of speed) as an example trait. If Greyhounds were selected for breeding based solely on genetic merit for race time or speed, however, this may in fact be selecting for dogs with lighter and weaker bones and therefore increased risk of injury. In future work, genetic evaluations could be performed and EBVs calculated for other Greyhound health, welfare and economically-important traits where recorded data is available, such as injury and disease resistance, longevity, trainability, behavioural traits and adaptation to a kennel environment. The resulting EBVs could be combined with those for speed and race time and used to create a selection index, providing a useful tool for breeders to use in the selection of Greyhounds for breeding. The application of such a selection index to Greyhound breeding practices would enable the simultaneous genetic improvement in a number of traits within the population.

Recent scientific advances in the area of animal breeding, and development of next-generation sequencing and genotyping technologies, have enabled the estimation of breeding value based on a combination of phenotypic measurements of performance, pedigree information and genome sequencing data, to produce a Genomic Estimated Breeding Value (GEBV) (Eggen, 2012). Advantages of selection based on GEBVs compared to selection based on traditional EBVs include the potential for improved accuracy and a considerably

faster rate of genetic improvement (Goddard and Hayes, 2009). In future studies, genotyping of a large reference population of racing Greyhounds could be performed to identify polymorphisms associated with complex performance traits such as Race Time or Speed, and used in combination with the phenotypic performance and pedigree data to create GEBVs to further assist in selection of animals for breeding. Additionally, if phenotypic injury data were made available for Greyhounds racing in the UK, it may be possible in future studies to combine this with genotyping data, in order to produce GEBVs for the risk of stress fracture and other serious racing injuries.

One of the challenges faced by the Greyhound industry and researchers is changing the views of Greyhound breeders, owners and trainers on traditional methods of breeding that have been employed since the introduction of Greyhound racing as a sport. As a direct result of the work carried out in this thesis, the GBGB established the Breeding Advisory Group for Racing Animals (BAGRA) in January 2015. The initial aims of BAGRA include educating members of the Greyhound industry in the requirement for a move from traditional methods of breeding to methods based on genetic analysis, and to identify and organise areas of further research. As a large proportion (75%) of Greyhounds that race in the UK were originally bred in Ireland (APGAW, 2007), fully addressing the issue of over-breeding will require collaboration between the Irish and British Greyhound industries. The research conducted in this thesis has therefore triggered the start of change within the Greyhound industry. This can now be taken forward and

expanded upon in order to further investigate and develop methods of targeted genetic selection for Greyhound breeding.

7.3.3. Investigation of Genetic Factors in the Pathogenesis of Stress Fracture Injuries in the Racing Greyhound

This thesis presents the first genetic investigation of stress fracture injuries in the racing Greyhound. A DNA archive was established for British racing Greyhounds, providing a valuable resource for future genetic research. A total of 237 Greyhound DNA samples (135 males and 102 females) from Greyhounds that had sustained a stress fracture ($n = 91$ dogs) and from healthy uninjured dogs ($n = 146$ dogs) were obtained from Greyhound saliva, skin and muscle tissue samples. For each individual in the archive, detailed pedigree, phenotypic, race history and injury data was collected. Previous canine GWA studies have frequently made use of control DNA samples obtained from DNA archives, usually with very little phenotypic data available for the individual animal (Massey *et al.*, 2014; Wood *et al.*, 2009). Each Greyhound sample in the DNA archive established in the present study, however, was extremely well phenotyped. This provides a useful resource for future validation of the results obtained in the thesis, as well as for further genetic studies of the racing Greyhound.

A discovery GWA study was performed to investigate genetic factors that may predispose racing Greyhounds to develop specific stress fracture injuries. Genome-

wide SNP genotyping of 21 case (affected) and 24 control (unaffected) Greyhound DNA samples was performed using the Illumina® CanineHD BeadChip (Illumina, San Diego, California, USA). No SNPs were found to reach genome-wide statistical significance at the $p \leq 0.00001$ significance level, or remained significant after Bonferroni correction, and this is likely to be due to the small sample size used in the study. For further investigation of SNPs associated with stress fracture risk, larger sample sizes would be required. A further limitation of the GWA study design was the inclusion of second-degree relatives, due to the limited availability of DNA samples from unrelated individuals. This may introduce bias, as particular within-family genotypes are over-represented in the study (Anderson *et al.*, 2010). All Greyhounds included in the study were extremely well phenotyped, however, with detailed pedigree, race history and injury data obtained for both case and control individuals.

As an initial discovery GWAS, the study has identified a number of nominally associated SNPs and regions of interest on chromosomes that may be implicated in the pathogenesis of stress fractures in the racing Greyhound, and that warrant further investigation. 11 SNPs were significantly associated at the $p \leq 0.0001$ level and a further 96 SNPs at the $p \leq 0.001$ level. Interestingly, several of the most significantly associated polymorphisms were identified within or in close proximity to genes involved in bone metabolism pathways. This included: the PPARG gene, a key modulator of skeletal remodelling and bone homeostasis; SFRP2, a regulator of cell differentiation and bone formation; PPP2R2D, an important negative modulator of the TGF- β (transforming growth factor beta) signalling pathway

involved in the maintenance and development of the progenitors of osteoblasts and bone formation; and ATP6V1C2, involved in osteoclastic bone resorption.

This study is the first to investigate and identify SNPs that are nominally associated with stress fracture risk in the racing Greyhound. Further work is required to validate the associations by fine-mapping using a larger cohort of case and control samples from the same Greyhound population. Targeted sequencing of the regions of interest is then required, in order to identify the causative mutations and specific genetic risk factors for the development of Greyhound stress fracture injuries.

Stress fracture is thought to be a multifactorial multigenic disorder controlled by both genetic and environmental factors (Yanovich *et al.*, 2012). Further studies could investigate whether known environmental risk factors in humans are also involved in the pathogenesis of stress fracture in the racing Greyhound, such as training intensity, training surface, nutrition, gender, age, height, type of physical activity, fitness level, serum parathyroid concentration, serum vitamin D concentration and bone mineral density. Additional factors such as body weight, frequency of racing, race distance, number of race starts and mean racing speed could also be investigated.

Once causative genetic mutations are confirmed, the results can be used to inform selection of Greyhounds for breeding in order to produce stronger racing animals that are less predisposed to injury. The additional identification of environmental

risk factors could be used to inform Greyhound nutrition and management, for example, in order to minimise the overall risk of stress fracture injury.

In addition, further work could investigate SNPs associated with other complex traits in Greyhounds such as longevity, susceptibility to disease, trainability and behavioural traits.

7.3.4. Salivary Cortisol and Heart Rate as Measures of Stress in the Racing Greyhound

Finally, this thesis investigated the effects of exercise and a number of environmental factors on cortisol and HR indicators of stress in the racing Greyhound. The thesis identified that short-duration high-intensity sprint exercise in Greyhounds results in a statistically significant increase in both HR and secretion of the glucocorticoid hormone cortisol, which is detectable in saliva samples immediately post-racing. There were significant differences pre-race and post-race between each Greyhound's paired salivary log cortisol concentration ($p = 0.000004$) and paired HR ($p = 0.002$). For both salivary log cortisol concentration and HR, the post-race values were significantly higher than pre-race values. Furthermore, the mean Greyhound salivary cortisol concentration pre-race (12.17 ng/ml) was considerably higher than reported baseline values in dogs (0.8 ng/ml to 5.3 ng/ml) (Glenk *et al.*, 2013; Stracke *et al.*, 2011; Vincent and Michell, 1992), indicating increased arousal and activation of the HPA axis during the immediate

pre-race period. There was no correlation between Greyhound salivary log cortisol concentration and HR in pre-race ($R^2 = 0.0098$) or post-race ($R^2 = 0.0104$) measurements. Greyhound sex, racing age, journey distance to the stadium, race distance and rank finish position in the race were not associated ($p > 0.05$) with pre- or post-race salivary log cortisol concentration or HR.

A limitation of the study is that resting salivary cortisol concentration and HR were not measured. Consequently, the pre- and post-race cortisol levels and HR cannot be compared to basal values in the Greyhounds studied. Further work is required to establish basal cortisol concentrations and HR for Greyhounds on non-race days, as well as measurements before, during and after transport to the stadium on the day of the race. In addition, due to a delay of up to five minutes between the end of the race and HR measurement, the post-race HR data collected in the study may not be a true indication of the immediate post-race values. Future studies could consider measures of HR variability using a continuous HR monitoring system (Becker-Birck *et al.*, 2013; Lewinski *et al.*, 2013; Schmidt *et al.*, 2010a,b,c). Alternative or additional measures of stress could also be investigated, such as blood pressure, gastrointestinal activity, immunological changes and behaviour (Bergeron *et al.*, 2002; Hekman *et al.*, 2012; Kay and Hall, 2009; Menor-Campos *et al.*, 2011; Moberg, 2000; Ottenheimer Carrier *et al.*, 2013; Pastore *et al.*, 2011).

The study took place at an Independent (non-GBGB) Greyhound stadium. There are many differences, however, between Independent and GBGB-licensed

Greyhounds, stadiums and race day procedures. Greyhounds racing at Independent tracks frequently live in the home as pet dogs, whereas licensed Greyhounds are required to live in a kennel environment. Transport to the race track may also differ, with some non-GBGB Greyhounds transported in cars rather than vans. Independent tracks are required to provide kennels for at least 20% of the Greyhounds racing on a given night. Use of the kennels is optional, however, and in the present study these kennels were never used. In contrast, all GBGB-licensed Greyhounds are required to be kennelled for at least 45 minutes prior to their race or trial. It would be useful in future work to investigate cortisol as a measure of stress in GBGB-licensed Greyhounds, to determine whether differences exist between the two sectors of racing dogs pre- and post-race. Future studies could investigate effects of the required pre-race kennelling period on measures of Greyhound stress. Additionally, the detailed performance data available for GBGB-licensed dogs would enable the investigation of associations between cortisol concentration and race performance.

This thesis has identified that sprint exercise in Greyhounds results in activation of the HPA axis and an increase in salivary cortisol concentration. The frequent increase in cortisol secretion as a result of regular racing may have implications for the health and welfare of the Greyhound, as frequent or chronic elevation of glucocorticoids is associated with protein catabolism, hyperglycaemia, immunosuppression and reduced reproductive performance (Dobson and Smith, 1995; Matteri *et al.*, 2000; Mostl and Palme, 2002). Furthermore, increased levels of glucocorticoids are reported to increase the risk of osteoporosis and bone

fracture in humans (Hardy and Cooper, 2010). Future studies could investigate the long-term effects of frequent cortisol elevation on Greyhound health and welfare, and the influence of cortisol levels in the development of stress fracture injuries. In addition, an individual's response to stressful stimuli is controlled by both genetic and environmental factors (Pottinger, 2000). Further studies could investigate the genetic factors associated with a Greyhound's stress response and ability to cope with potentially stressful stimuli such as racing, kennelling and transport.

7.3.5. Conclusions

Overall, this thesis has identified a number of environmental (non-genetic) factors that affect British Greyhound race performance. Variance components, estimated heritabilities and repeatabilities have been calculated for the industry-important performance traits Race Time, Speed and Rank, and EBVs have been calculated for 73,344 British Greyhounds. In addition, this thesis has identified a number of nominally associated SNPs and regions of interest on chromosomes that may be implicated in the pathogenesis of stress fracture injuries in the racing Greyhound, and that warrant further investigation. Furthermore, this thesis has identified that short-duration, high-intensity sprint exercise in Greyhounds results in a significant increase in HR and salivary cortisol concentration, detectable immediately post-race. The results of this work, along with further studies, can be used to inform future Greyhound breeding practices in order to produce stronger, less injury-prone and better-performing dogs of predictable and superior genetic merit. Importantly, the responsible use of targeted genetic selection within the industry may improve Greyhound welfare by minimising the number of surplus dogs produced.

References

- Accorsi, P.A., Carloni, E., Valsecchi, P., Viggiani, R., Gamberoni, M., Tamanini, C. & Seren, E. (2008) 'Cortisol determination in hair and faeces from domestic cats and dogs', *General and Comparative Endocrinology*, 155, pp.398-402.
- Ackert-Bicknell, C.L., Demissie, S., Marin de Evsikova, C., Hsu, Y.H., DeMambro, V.E., Karasik, D., Cupples, L.A., Ordovas, J.M., Tucker, K.L., Cho, K., Canalis, E., Paigen, B., Churchill, G.A., Forejit, J., Beamer, W.G., Ferrari, S., Bouxsein, M.L., Kiel, D.P. & Rosen, C.J. (2008) 'PPARG by dietary fat interaction influences bone mass in mice and humans', *Journal of Bone and Mineral Research*, 23 (9), pp.1398-1408.
- Agriculture and Horticulture Development Board. (2014) *AHDB Dairy Breeding and Genetics* [Online] Available from: https://dairy.ahdb.org.uk/technical-information/breeding-genetics/#.WNwAg_krJPY (Accessed 11 January, 2016).
- Alam, I., Sun, Q., Koller, D.L., Liu, L., Liu, Y., Edenberg, H.J., Li, J., Foroud, T. & Turner, C.H. (2009) 'Differentially expressed genes strongly correlated with femur strength in rats', *Genomics*, 94, pp.257-262.
- Alberts, B; Johnson, A., Lewis, J., Raff, M., Roberts, K. & Walker, P. (2002) 'An Overview of Gene Control'. In: *Molecular Biology of the Cell*. 4th ed. New York: Garland Science.
- Aldridge, L.I., Kelleher, D.L., Reilly, M. & Brophy, P.O. (2000) 'Estimation of the genetic correlation between performances at different levels of show jumping competitions in Ireland', *Journal of Animal Breeding and Genetics*, 117 (1), pp.65-72.

Alfaro, M.P., Vincent, A., Saraswati, S., Thorne, C.A., Hong, C.C., Lee, E. & Young, P. (2010) 'sFRP2 Suppression of Bone Morphogenic Protein (BMP) and Wnt Signaling Mediates Mesenchymal Stem Cell (MSC) Self-renewal Promoting Engraftment and Myocardial Repair', *Journal of Biological Chemistry*, 285 (46), pp.35645-35653.

Altmuller, J., Palmer, L.J., Fischer, G., Scherb, H. & Wjst, M. (2001) 'Genomewide scans of complex human diseases: true linkage is hard to find', *American Journal of Human Genetics*, 69, pp.936-950.

Amer, P.R., Crump, R. & Simm, G. (1998) 'A terminal sire selection index for UK beef cattle', *Animal Science*, 67 (3), pp.445-454.

Amer, P.R., Nieuwhof, G.J., Pollott, G.E., Roughsedge, T., Conington, J. & Simm, G. (2007) 'Industry benefits from recent genetic progress in sheep and beef populations', *Animal*, 1 (10), pp.1414-1426.

American Kennel Club. (1998) *American Kennel Club*. [Online] Available from: <http://www.akc.org/> (Accessed 1 January 2016).

Amphlett, F. (1993) *In the Blood - Complete Guide to Greyhound Breeding*. Bath: Fenman Books.

Anderson, C.A., Pettersson, F.H., Clarke, G.M., Cardon, L.R., Morris, A.P. & Zondervan, K.T. (2010) 'Data quality control in genetic case-control association studies', *Nature Protocols*, 5 (9), pp.1564-1573.

Anderson, M.A., Constantinescu, G.M., Dee, L.G. & Dee, J.F. (1995) 'Fractures and dislocations of the racing greyhound - Part II', *The Compendium for Continuing Education of Practicing Veterinarians*, 17, pp.899-909.

Andersson, L. (2001) 'Genetic dissection of phenotypic diversity in farm animals', *Nature Reviews Genetics*, 2, February, pp.130-138.

Andersson, L. (2009) 'Genome-wide association analysis in domestic animals: a powerful approach for genetic dissection of trait loci', *Genetica*, 136, pp.341-349.

Angle, C.T., Wakshlag, J.J., Gillette, R.L., Stokol, T., Geske, S., Adkins, T.O. & Gregor, C. (2009) 'Hematologic, serum biochemical, and cortisol changes associated with anticipation of exercise and short duration high-intensity exercise in sled dogs', *Veterinary Clinical Pathology*, 38 (3), pp.370-374.

Angle, T.C., Gillette, R.L. & Weimar, W.H. (2012) 'Caudal paw displacement during movement initiation and its implications for possible injury mechanisms', *Veterinary and Comparative Orthopaedics and Traumatology*, 5, pp.397-401.

Animal Welfare Act 2006. (c.45) London: HMSO.

APGAW (Associate Parliamentary Group for Animal Welfare). (2007) *The welfare of greyhounds: Report of the APGAW inquiry into the welfare issues surrounding racing greyhounds in England*. May 2007.

APGAW (Associate Parliamentary Group for Animal Welfare). (2009) *A Healthier Future for Pedigree Dogs*. November 2009.

APGAW (Associate Parliamentary Group for Animal Welfare). (2012) *A Healthier Future for Pedigree Dogs Update Report*. July 2012.

Armstrong, R.B., Saubert, S.W., Seeherman, J.J. & Taylor, C.R. (1982) 'Distribution of fiber types in locomotory muscles of dogs', *The American Journal of Anatomy*, 163, pp.87-98.

Árnason, T. (2001) 'Trends and asymptotic limits for racing speed in standardbred trotters', *Livestock Production Science*, 72, pp.135-145.

Arvelius, P. & Klemetsdal, G. (2013) 'How Swedish breeders can substantially increase the genetic gain for the English Setter's hunting traits', *Journal of Animal Breeding and Genetics*, 130, pp.142-153.

Baird, A.E.G., Carter, S.D., Innes, J.F., Ollier, W. & Short, A. (2014) 'Genome-wide association study identifies genomic regions of association for cruciate ligament rupture in Newfoundland dogs', *Animal Genetics*, 45, pp.542-549.

Bakhtiari, J. & Kashan, N.E.J. (2009) 'Estimation of genetic parameters of racing performance in Iranian Thoroughbred horses', *Livestock Science*, 120, pp.151-157.

Baron, R. & Kneissel, M. (2013) 'WNT signaling in bone homeostasis and disease: from human mutations to treatments', *Nature Medicine*, 19, pp.179-192.

Barrett, J.C., Fry, B., Maller, J. & Daly, M.J. (2005) 'Haploview: analysis and visualization of LD and haplotype maps', *Bioinformatics*, 21 (2), pp.263-265.

Barrey, E. (2010) 'Genetics and genomics in equine exercise physiology: an overview of the new applications of molecular biology as positive and negative markers of performance and health', *Equine Veterinary Journal*, 42 (Suppl. 38), pp.561-568.

Bastin, C., Soyeurt, H. & Gengler, N. (2013) 'Genetic parameters of milk production traits and fatty acid contents in milk for Holstein cows in parity 1 – 3', *Journal of Animal Breeding and Genetics*, 130, pp.118-127.

Bateman, J.K. (1960) 'The racing greyhound', *Veterinary Record*, 72, pp.893-897.

Bateson, P. (2010) *Independent Inquiry into Dog Breeding*. December 2009.

Batut, J., Schmierer, B., Cao, J., Raftery, L.A., Hill, C.S. & Howell, M. (2008) 'Two highly related regulatory subunits of PP2A exert opposite effects on TGF- β /Activin/Nodal signalling', *Development*, 135, pp.2927-2937.

Becker, A.C., Stock, K.F. & Distl, O. (2011) 'Genetic correlations between free movement and movement under rider in performance tests of German Warmblood horses', *Livestock Science*, 142, pp.245-252.

Becker-Birck, M., Schmidt, A., Lasarzik, J., Aurich, J., Möstlk, E. & Aurich, C. (2013) 'Cortisol release and heart rate variability in sport horses participating in equestrian competitions', *Journal of Veterinary Behavior*, 8, pp.87-94.

Beerda, B., Schilder, M.B.H., Janssen, N.S.R.M. & Mol, J.A. (1996) 'The Use of Saliva Cortisol, Urinary Cortisol, and Catecholamine Measurements for a Noninvasive Assessment of Stress Responses in Dogs', *Hormones and Behavior*, 30, pp.272-279.

Beerda, B., Schilder, M.B.H., Hooff, J.A.R.A.M. van, Vries, H.W. de & Mol, J.A. (1998) 'Behavioural, saliva cortisol and heart rate responses to different types of stimuli in dogs', *Applied Animal Behaviour Science*, 58, pp.365-381.

Bellenger, C.R., Johnson, K.A., Davis, P.E. & Ilkiw, J.E. (1981) 'Fixation of metacarpal and metatarsal fractures in greyhounds', *Australian Veterinary Journal*, 57, May, pp.205-211.

Bennell, K., Matheson, G., Meeuwisse, W. & Brukner, P. (1999) 'Risk Factors for Stress Fractures', *Sports Medicine*, 28 (2), pp.91-122.

Bentall, H. (2008) 'Deaths in racing greyhounds', *Veterinary Record*, 162, pp.254-255.

Bergamasco, L., Osella, M.C., Savarino, P., Larosa, G., Ozella, L., Manassero, M., Odore, R., Barbero, R. & Re, G. (2010) 'Heart rate variability and saliva cortisol assessment in shelter dog: Human–animal interaction effects', *Applied Animal Behaviour Science*, 125, pp.56-68.

Bergeron, R., Scott, S.L., Émond, J., Mercier, F., Cook, N.J. & Schaefer, A.L. (2002) 'Physiology and behavior of dogs during air transport', *The Canadian Journal of Veterinary Research*, 66, pp.211-216.

Bergh, M.S., Piras, A., Samii, V.F., Weisbrode, S.E. & Johnson, K.A. (2012) 'Fractures in regions of adaptive modeling and remodeling of central tarsal bones in racing Greyhounds', *American Journal of Veterinary Research*, 73 (3), March, pp.375-380.

Betting and Gaming Act 1960. London: HMSO.

Biddle, S.T.B., O'Donnell, A., Houghton, E. & Creaser, C.S. (2013) 'Metabolism of norethisterone in the greyhound', *Rapid Communications in Mass Spectrometry*, 27, pp.2229-2238.

Bloomberg, M.S. (1989) 'Racing greyhound track injuries', In: *Proc. Int. Racing Greyhound Symposium*, Florida, pp.3-12.

Bloomberg, M.S. (1995) 'Racing injuries of greyhounds: Racetrack injury survey', In: *Proc. 11th Ann. Int. Canine Sports Medicine Symposium*, Florida, pp.49-55.

Blythe, L.L., Gannon, J.R., Craig, A.M. & Fegan, D.P. (2007) *Care of the Racing & Retired Greyhound*. 1st ed. Kansas, USA: Hall Commercial Printing.

Boemo, C.M. & Eaton-Wells, R.D. (1995) 'Medial displacement of the tendon of origin of the biceps brachii muscle in 10 greyhounds', *Journal of Small Animal Practice*, 36, pp.69-73.

Bokor, A., Blouin, C. & Langlois, B. (2007) 'Possibility of selecting racehorses on jumping ability based on their steeplechase race results in France, the United Kingdom and Ireland', *Journal of Animal Breeding and Genetics*, 124, pp.124-132.

Bord na gCon. (2014) *Bord na gCon (Irish Greyhound Board)*. [Online] Available from: <http://www.igb.ie> (Accessed: 10 January 2014).

Borell, E. von, Dobson, H. & Prunier, A. (2007) 'Stress, behaviour and reproductive performance in female cattle and pigs', *Hormones and Behavior*, 52, pp.130-138.

Bormann, J. Minick & Wilson, D.E. (2010) 'Calving day and age at first calving in Angus heifers', *Journal of Animal Science*, 88, pp.1947-1956.

Boudrieau, R.J., Dee, J.F. & Dee, L.G. (1984a) 'Central tarsal bone fractures in the racing Greyhound: a review of 114 cases', *Journal of the American Veterinary Medical Association*, 184 (2), pp.1486-1491.

Boudrieau, R.J., Dee, J.F. & Dee, L.G. (1984b) 'Treatment of central tarsal bone fractures in the racing greyhound', *Journal of the American Veterinary Medical Association*, 184, pp.1492-1500.

Bouquet, A., Fouilloux, M.-N., Renand, G. & Phocas, F. (2010) 'Genetic parameters for growth, muscularity, feed efficiency and carcass traits of young beef bulls', *Livestock Science*, 129, pp.38-48.

Bourdon, R.M. (2014) *Understanding Animal Breeding*. 2nd ed. Essex: Pearson Education Ltd.

Boyko, A.R. (2011) 'The domestic dog: man's best friend in the genomic era', *Genome Biology*, 12, Article Number 216.

Brambell Report. (1965). *Report of the Technical Committee to enquire into the welfare of animals kept under intensive livestock husbandry systems*. Her Majesty's Stationary Office, London, UK.

Breeding and Sale of Dogs (Welfare) Act 1999. (c.11) London: HMSO.

Breur, G.J., Lambrechts, N.E. & Todhunter, R.J. (2012) 'Chapter 7: The Genetics of Canine Orthopaedic Traits'. In: Ostrander, E.A. & Ruvinsky, A. (eds.) *The Genetics of the Dog*. 2nd ed. Oxfordshire: CABI Publishing.

British Greyhound Racing Fund (BGRF). (2012) *British Greyhound Racing Fund (BGRF)*. [Online] Available from: <http://www.bgrf.org.uk> (Accessed 20 July 2012).

British Greyhound Racing Fund (BGRF). (2014) *British Greyhound Racing Fund Annual Reports & Accounts 2013-14*. British Greyhound Racing Fund Limited. London.

British Greyhound Racing Fund (BGRF). (2015) *British Greyhound Racing Fund Annual Reports & Accounts 2014-15*. British Greyhound Racing Fund Limited. London.

British Veterinary Association (BVA). (2014) *British Veterinary Association*. [Online] Available from: <http://www.bva.co.uk/Canine-Health-Schemes/> (Accessed: 8 January 2014).

Broom, D.M. & Johnson, K.G. (1993) *Stress and Animal Welfare*. London: Chapman & Hall.

Brorsson, C., Dahlqvist, P., Nilsson, L. & Naredi, S. (2014) 'Saliva stimulation with glycerine and citric acid does not affect salivary cortisol levels', *Clinical Endocrinology*, 81, pp.244-248.

Brown, C.M. (1986) *Dog Locomotion and Gait Analysis*. Colorado: Hoflin.

Brukner, P., Bradshaw, C., Khan, K.M., White, S. & Crossley, K. (1996) 'Stress Fractures: A review of 180 cases', *Clinical Journal of Sport Medicine*, 6 (2), pp.85-89.

Bruns, E., Bade, B. & Haring, H. (1980) 'Results on a more objective measurement of performance traits of stallions in performance testing at station', *Livestock Production Science*, 7, pp.607-614.

Bryan, H.M., Adams, A.G., Invik, R.M., Wynne-Edwards, K.E. & Smits, E.G.J. (2013) 'Hair as a Meaningful Measure of Baseline Cortisol Levels over Time in Dogs', *Journal of the American Association for Laboratory Animal Science*, 52 (2), March, pp.189-196.

Bugislaus, A.-E., Roehe, R., Uphaus, H. & Kalm, E. (2004) 'Development of genetic models for estimation of racing performances in German thoroughbreds', *Archiv Tierzucht*, 47, pp.505-516.

Bugislaus, A.-E., Roehe, R. & Kalm, E. (2005) 'Comparison of two different statistical models considering individual races or racetracks for evaluation of German trotters', *Livestock Production Science*, 92, pp.69-76.

Bugislaus, A.-E. (2010) 'Genetic analysis of distance-dependent racing performances in German Thoroughbreds', *Archiv Tierzucht*, 53 (6), pp.629-638.

Buono, M.J., Yeager, J.E. & Hodgdon, J.A. (1986) 'Plasma adrenocorticotropin and cortisol responses to brief high-intensity exercise in humans', *Journal of Applied Physiology*, 61 (4), pp.1337-1339.

Burr, D.B., Martin, R.B., Schaffler, M.B. & Radin, E.L. (1985) 'Bone remodeling in response to in vivo fatigue microdamage', *Journal of Biomechanics*, 18 (3), pp.189-200.

Buttchereit, N., Stamer, E., Junge, W. & Thaller, G. (2012) 'Genetic parameters for energy balance, fat to protein ratio, body condition score and disease traits in German Holstein cows', *Journal of Animal Breeding and Genetics*, 129, pp.280-288.

Buttram, S.T., Willham, R.L. & Wilson, D.E. (1988) 'Genetics of Racing Performance in the American Quarter Horse: II. Adjustment Factors and Contemporary Groups', *Journal of Animal Science*, 66, pp.2800-2807.

Buxadera, A.M. & Mota, M.D.S. da (2008) 'Variance component estimations for race performance of thoroughbred horses in Brazil by random regression model', *Livestock Science*, 117, pp.298-307.

Calboli, F.C.F., Sampson, J., Fretwell, N. & Balding, D.J. (2008) 'Population Structure and Inbreeding From Pedigree Analysis of Purebred Dogs', *Genetics*, 179, May, pp.593-601.

Campora, C., Freeman, K.P., Lewis, F.I., Sacchini, F. & Sanchez-Vasquez, M.J. (2011) 'Determination of haematological reference intervals in healthy adult greyhounds', *Journal of Small Animal Journal*, 52, June, pp.301-309.

CanFam1.0. (2004) Broad Institute of MIT and Harvard. July 2004 [Online] Available from: <https://www.broadinstitute.org/disease-research/dog-snps-canfam-10> (Accessed February 1, 2012).

CanFam2.0. (2005) Broad Institute of MIT and Harvard. May 2005 [Online] Available from: <https://www.broadinstitute.org/disease-research/dog-snps-canfam-20> (Accessed February 1, 2012).

CanFam3.1. (2011) Broad Institute of MIT and Harvard. September 2011 [Online] Available from: <ftp://ftp.broadinstitute.org/distribution/assemblies/mammals/dog/canFam3.1/> (Accessed February 1, 2012)

Cannon, W.B. (1929) *Bodily Changes in Pain, Hunger, Fear and Rage: An account of Recent Researches into the Function of Emotional Excitement*. Appleton, New York.

Carta, A., Casi, S. & Salaris, S. (2009) 'Invited review: Current state of genetic improvement in dairy sheep', *Journal of Dairy Science*, 92 (12), pp.5814-5833.

Chang, M.L., Terrill, R.L., Bautista, M.M., Carlson, E.J., Dyer, D.J., Overall, K.L. & Hamilton, S.P. (2007) 'Large-Scale SNP Genotyping with Canine Buccal Swab DNA', *Journal of Heredity*, 98 (5), pp.428-437.

Chase, K., Carrier, D.R., Adler, F.R., Jarvik, T., Ostrander, E.A., Lorentzen, T.D. & Lark, K.G. (2002) 'Genetic basis for systems of skeletal quantitative traits: Principal component analysis of the canid skeleton', *Proceedings of the National Academy of Sciences of the United States of America*, 99 (15), July, pp.9930-9935.

Chatzipapas, C., Boikos, S., Drosos, G.I., Kazakos, K., Tripsianis, G., Serbis, A., Stergiopoulos, S., Tilkeridis, C., Verettas, D.A. & Stratakis, C.A. (2009) 'Polymorphisms of the Vitamin D Receptor Gene and Stress Fractures', *Hormone and Metabolic Research*, 41 (8), pp.635-640.

Chen, G., Deng, C. & Li, Y-P. (2012) 'TGF- β and BMP Signaling in Osteoblast Differentiation and Bone Formation', *International Journal of Biological Sciences*, 8 (2), pp.272-288.

Cheney, J.A., Shen, C.K. & Wheat, J.D. (1973) 'Relationship of racetrack surface to lameness in the thoroughbred racehorse', *American Journal of Veterinary Research*, 34, pp.1285-1289.

Chico, M.D. (1994) 'Genetic analysis of Thoroughbred racing performance in Spain', *Annales de Zootechnie*, 43, pp.393-397.

Chuckpaiwong, B., Cook, C., Pietrobon, R. & Nunley, J.A. (2007) 'Second metatarsal stress fracture in sport: comparative risk factors between proximal and non-proximal locations', *British Journal of Sports Medicine*, 41, pp.510-514.

Clarke, H.E. (1980) *The Greyhound*. 7th ed. London: Popular Dogs Publishing Co. Ltd.

Clarke, G.M., Anderson, C.A., Pettersson, F.H., Cardon, L.R., Morris, A.P. & Zondervan, K.T. (2011) 'Basic statistical analysis in genetic case-control studies', *Nature Protocols*, 6 (2), pp.121-133.

Cline, A.D., Jansen, G.R. & Melby, C.L. (1998) 'Stress Fractures in Female Army Recruits: Implications of Bone Density, Calcium Intake, and Exercise', *Journal of American College Nutrition*, 17 (2), pp.128-135.

Cook, A. (1998) 'Literature survey of racing greyhound injuries, performance and track conditions', *Journal of Turfgrass Science*, 74, pp.108-113.

Corbin, L.J., Blott, S.C., Swinburne, J.E., Sibbons, C., Fox-Clipsham, L.Y., Helwegen, M., Parkin, T.D.H., Newton, J.R., Bramlage, L.R., Mellwraith, C.W., Bishop, S.C., Woolliams, J.A. & Vaudin, M. (2012) 'A genome-wide association study of osteochondritis dissecans in the Thoroughbred', *Mammalian Genome*, 23, pp.294-303.

Corrêa, M.J.M. & Mota, M.D.S da (2007) 'Genetic evaluation of performance traits in Brazilian Quarter Horse', *Journal of Applied Genetics*, 48 (2), pp.145-151.

Costa, R.B., Misztal, I., Elzo, M.A., Bertrand, J.K., Silva, L.O.C. & Lukaszewicz, M. (2011) 'Estimation of genetic parameters for mature weight in Angus cattle', *Journal of Animal Science*, 89, pp.2680-2686.

Council Regulation (EC) No 1/2005 of 22 December 2004 on the protection of animals during transport and related operations and amending Directives 64/432/EEC and 93/119/EC and Regulation (EC) No 1255/97. The Council of the European Union.

Cunningham, F., Amode, M.R., Barrell, D., Beal, K., Billis, K., Brent, S., Carvalho-Silva, D., Clapham, P., Coates, G., Fitzgerald, S., Gil, L., Girón, C.G., Gordon, L., Hourlier, T., Hunt, S.E., Janacek, S.H., Johnson, N., Juettemann, T., Kähäri, A.K., Keenan, S., Martin, F.J., Maurel, T., McLaren, W., Murphy, D.N., Nag, R., Overduin, B., Parker, A., Patricio, M., Perry, E., Pignatelli, M., Riat, H.S., Sheppard, D., Taylor, K., Thormann, A., Vullo, A., Wilder, S.P., Zadissa, A., Aken, B.L., Birney, E., Harrow, J., Kinsella, R., Muffato, M., Ruffier, M., M.J. Searle, S.M.J., Spudich, G., Trevanion, S.J., Yates, A., Zerbino, D.R. & Flicek, P. (2015) 'Ensembl', *Nucleic Acids Research*, 43 (D1). Database issue: D662-D669.

Davis, P.E. (1967) 'Track injuries in racing greyhounds', *Australian Veterinary Journal*, 43, May, pp.180-191.

Davis, P.E. (1973) 'Toe and muscle injuries of the racing greyhound', *New Zealand Veterinary Journal*, 21 (7), pp.133-146.

Dee, J.F. (1998) *Tarsal Injuries*. In: Canine Sports Medicine and Surgery. Eds M.S. Bloomberg, J.F. Dee & R.A. Taylor. Lea & Febiger, Philadelphia, pp.519-570.

Dee, J.F., Dee, J. & Piermattei, D.L. (1976) 'Classification, management and repair of central tarsal fractures in the racing greyhound', *Journal of the American Animal Hospital Association*, 12, pp.398-405.

Dee, J.F. & Dee, L.G. (1985) 'Fractures and Dislocations Associated with Racing Greyhound'. In: Newton, C.D. & Nunamaker, D.M. (eds.) *Textbook of Small Animal Orthopaedics*. Philadelphia: Lippincott, Williams and Wilkins, ch.35.

Dee, J.F., Dee L.G. & Eaton-Wells, R.D. (1990) *Injuries of high performance dogs*. In: Canine Orthopaedics. 2nd edn. Ed: W.G. Whittick. Philadelphia: Lea & Febiger, pp.532.

Delwiche, L.D. & Slaughter, S.J. (2012) *The Little SAS® Book: A Primer*. 5th ed. North Carolina: SAS Institute Inc.

Denny, M.W. (2008) 'Limits to running speed in dogs, horses and humans', *Journal of Experimental Biology*, 211, pp.3836-3849.

Department for Environment, Food and Rural Affairs. (2010) *Guidance for Independent greyhound track operators*. February 2010.

Desgorces, F.-D., Berthelot, G., Charmantier, A., Tafflet, M., Schaal, K., Jarne, P. & Toussaint, J.F. (2012) 'Similar slow down in running speed progression in species under human pressure', *Journal of Evolutionary Biology*, 25, pp.1792-1799.

Devas, M.B. (1961) 'Compression stress fractures in man and the greyhound', *The Journal of Bone and Joint Surgery*, 43B (3), August, pp.540-551.

Devas, M.B. (1967) 'Shin splints, or stress fractures of the metacarpal bone in horses, and shin soreness, or stress fractures of the tibia, in man', *The Journal of Bone and Joint Surgery*, 49B (2), May, pp.310-313.

Devas, M.B. (1969) 'Stress Fractures in Athletes', *Proceedings of the Royal Society of Medicine*, 62, September, pp.933-937.

Dittrich, N., Lucas, R. Dantas de, Maioral, M. F., Diefenthaler, F. & Guglielmo, L. G. A. (2013) 'Continuous and intermittent running to exhaustion at maximal lactate steady state: Neuromuscular, biochemical and endocrinal responses', *Journal of Science and Medicine in Sport*, 16, pp.545-549

Dobson, G.P., Parkhouse, W.S., Weber, J.M., Stuttard, E., Harman, J., Snow, D.H. & Hochachka, P.W. (1988) 'Metabolic changes in skeletal muscle and blood of greyhounds during 800-m track sprint', *The American Journal of - Physiology Regulatory, Integrative and Comparative Physiology*, 255, pp.513-529.

Dobson, H. & Smith, R.F. (1995) 'Stress and reproduction in farm animals', *Journal of Reproduction and Fertility. Supplement*, 49, pp.451-461.

Donoghue, B. (2007) *Independent Review of the Greyhound Industry in Great Britain*. November 2007.

Dragojevic, J., Ostanek, B., Mencej-Bedrac, S., Komadina, R., Prezelj, J. & Marc, J. (2011) 'PPARG gene promoter polymorphism is associated with non-traumatic hip fracture risk in the elderly Slovenian population: A pilot study', *Clinical Biochemistry*, 44, pp.1085-1089.

Dreschel, N.A. & Granger, D.A. (2009) 'Methods of collection for salivary cortisol measurement in dogs', *Hormones and Behavior*, 55, pp.163-168.

Drögemüller, C., Karlsson, E.K., Hytönen, M.K., Perloski, M., Dolf, G., Sainio, K., Lohi, H., Lindblad-Toh, K. & Leeb, T. (2008) 'A Mutation in Hairless Dogs Implicates FOXI3 in Ectodermal Development', *Science*, 321, September, pp.1462.

Ducro, B.J., Koenen, E.P.C., Tartwijk, J.M.F.M van & Bovenhuis, H. (2007) 'Genetic relations of movement and free-jumping traits with dressage and show-jumping performance in competition of Dutch Warmblood horses', *Livestock Science*, 07, pp.227-234.

Dubois, C., Manfredi, E. & Ricard, A. (2008) 'Optimization of breeding schemes for sport horses', *Livestock Science*, 118, pp.99-112.

DUKES (Digest of United Kingdom Weather Statistics) (2008 to 2012) Department of Energy and Climate Change. [Online] Available from: <https://www.gov.uk/government/statistics/weather-digest-of-united-kingdom-energy-statistics-dukes> (Accessed 1 September 2014).

Duncan, E.M. & Brown, M.A. (2010) 'Mapping genes for osteoporosis—Old dogs and new tricks', *Bone*, 46, pp.1219-1225.

Duncan, I.J.H. & Fraser, D. (1997). *Understanding Animal Welfare*. In: *Animal Welfare*. Eds: M.A. Appleby, B.O. Hughes. Wallingford, UK: CABI Publishing.

Durocher, L.L., Hinchcliff, K.W., Williamson, K.K., McKenzie, E.C., Holbrook, T.C., Willard, M., Royer, C.M. & Davis, M.S. (2007) 'Effect of strenuous exercise on urine concentrations of homovanillic acid, cortisol, and vanillylmandelic acid in sled dogs', *American Journal of Veterinary Research*, 68 (1), January, pp.107-111.

Eaglen, S.A.E., Coffey, M.P., Woolliams, J.A. & Wall, E. (2012) 'Evaluating alternate models to estimate genetic parameters of calving traits in United Kingdom Holstein-Friesian dairy cattle', *Genetics Selection Evolution*, 44 (23), pp.1-13.

Eggen, A. (2012) 'The development and application of genomic selection as a new breeding paradigm', *Animal Frontiers*, 2 (1), pp.10-15.

Ekiz, B. & Koçak, Ö. (2005) 'Phenotypic and genetic parameter estimates for racing traits of Arabian horses in Turkey', *Journal of Animal Breeding and Genetics*, 122, pp.249-356.

Ekiz, B. & Koçak, Ö. (2007) 'Estimates of Genetic Parameters for Racing Times of Thoroughbred Horses', *Turkish Journal of Veterinary and Animal Sciences*, 31 (1), pp.1-5.

Ekiz, B., Koçak, Ö. & Demir, H. (2005a) 'Estimates of Genetic Parameters for Racing Performances of Arabian Horses', *Turkish Journal of Veterinary and Animal Sciences*, 29, pp.543-549.

Ekiz, B., Koçak, Ö. & Yilmaz, A. (2005b) 'Phenotypic and Genetic Parameter Estimates for Racing Traits of Thoroughbred Horses in Turkey', *Archiv Tierzucht*, 48 (2), pp.121-129.

Emmerson, T.D., Lawes, T.J., Goodship, A.E., Rueux-Mason, C. & Muir, P. (2000) 'Dual-energy X-ray absorptiometry measurement of bone-mineral density in the distal aspect of the limbs in racing Greyhounds', *American Journal of Veterinary Research*, 61 (10), October, pp.1214-1219.

Engeland, W.C. & Gann, D.S. (1989) *Adrenal medullary and adrenal cortisol secretory responses to haemorrhage and to acoustic stimuli in awake dogs*. In: Stress; Neurochemical and Humoral Mechanisms. Eds G.R. Van Loom, R. Kvetnansky, R. McCarty & J. Axelrod. New York: Gordon & Breach. pp.613-625.

Ensembl. (2015) *Ensembl release 88, Dog (CanFam3.1)*. [Online] Available from: http://www.ensembl.org/Canis_familiaris/Info/Index (Accessed 6 January 2015).

Estrada, K., Styrkarsdottir, U., Evangelou, E., Hsu, Y.H. *et al.* (2012) 'Genome-wide meta-analysis identifies 56 bone mineral density loci and reveals 14 loci associated with risk of fracture', *Nature Genetics*, 44 (5), May, pp.491-503.

Evans, J.W. (1995) *Horses: A Guide to Selection, Care and Enjoyment*. 1st ed. W. H. Freeman Publishers.

Evans, H.E., de Lahunta, A. (2012) *Miller's Anatomy of the Dog*. 4th ed. St. Louis, Missouri: Saunders, Elsevier Inc.

Evans, R.K., Negus, C., Antczak, A., Yanovich, R., Israeli, E. & Moran, D. (2008) 'Sex Differences in Parameters of Bone Strength in New Recruits: Beyond Bone Density', *Official Journal of the American College of Sports Medicine*, 40 (11), November, pp.S645-S653.

Falconer, D.S. & Mackay, T.F.C. (1996) *Introduction to Quantitative Genetics*. 4th ed. Essex: Pearson Education Ltd.

Famula, T.R. (2012) 'Chapter 19: Genetics of Quantitative Traits and Improvement of the Dog Breeds'. In: Ostrander, E.A. & Ruvinsky, A. (eds.) *The Genetics of the Dog*. 2nd ed. Oxfordshire: CABI Publishing.

Farm Animal Welfare Council. (1979) *Farm Animal Welfare Council Press Statement*. December 5, 1979. Farm Animal Welfare Council, Surrey.

Farm Animal Welfare Council. (2009) *Farm Animal Welfare in Great Britain: Past, Present and Future*. October 2009. Farm Animal Welfare Council, London.

Fazio, E. & Ferlazzo, A. (2003) 'Evaluation of stress during transport', *Veterinary Research Communications*, 27 (Suppl. I), pp.519-524.

Fazio, E., Medica, P., Aronica, V., Grasso, L. & Ferlazzo, A. (2008a) 'Circulating β -endorphin, adrenocorticotrophic hormone and cortisol levels of stallions before and after short road transport: stress effect of different distances', *Acta Veterinaria Scandinavica*, 50 (6).

Fazio, E., Medica, P., Cravana, C. & Ferlazzo, A. (2008b) 'Effects of competition experience and transportation on the adrenocortical and thyroid responses of horses', *Veterinary Record*, 163, pp.713-716.

Fazio, E., Medica, P., Cravana, C. & Ferlazzo, A. (2013) 'Cortisol response to road transport stress in calm and nervous stallions', *Journal of Veterinary Behavior*, 8, pp.231-237.

Fels, L. & Distl, O. (2014) 'Identification and Validation of Quantitative Trait Loci (QTL) for Canine Hip Dysplasia (CHD) in German Shepherd Dogs', *PLOS One*, 9 (5), May.

Field., T.R.O., Murphy, J.W. & Lovejoy, P.J. (1993) 'Penetrometric assessment of the playability of course turf', *International Turfgrass Society Research Journal*, 7, pp.512-516.

Finestone, A. & Milgrom, C. (2008) 'How Stress Fracture Incidence Was Lowered in the Israeli Army: A 25-yr Struggle', *Official Journal of the American College of Sports Medicine*, 40 (11), November, pp.S623-S629.

Foggo, D. (2006) 'Revealed: the man who killed 10,000 dogs', *The Sunday Times*, July 16th 2006.

Fossum, T. Welch (2007) *Small Animal Surgery*. Missouri: Mosby Elsevier, pp.1233-1246.

Foyer, P., Bjallerhag, N. Wilson, E. & Jenson, P. (2014) 'Behaviour and experiences of dogs during the first year of life predict the outcome in a later temperament test', *Applied Animal Behaviour Science*, 155, pp.93-100.

French, A.T., Ogden, R., Eland, C., Hemani, G., Pong-Wong, R., Corcoran, B. & Summers, K.M. (2012) 'Genome-wide analysis of mitral valve disease in Cavalier King Charles Spaniels', *The Veterinary Journal*, 193, pp.283-286.

Friedl, K., Evans, R.K. & Moran, D.S. (2008) 'Stress Fracture and Military Medical Readiness: Bridging Basic and Applied Research', *Official Journal of the American College of Sports Medicine*, 40 (11), November, pp.S609-S622.

Friedl, K.E., Nuovo, J.A., Patience, T.H. & Dettori, J.R. (1992) 'Factors associated with stress fracture in young army women: indications for further research', *Military Medicine*, 157 (7), pp.334-338.

Gaffney, B. & Cunningham, E.P. (1988) 'Estimation of Genetic Trend in racing performance of thoroughbred horses', *Nature*, 332 (21), April, pp.722-724.

Galvez, R., Stacy, J. & Howley, A. (2008) 'Exertional Rhabdomyolysis in Seven Division-1 Swimming Athletes', *Clinical Journal of Sport Medicine*, 18 (4), July, pp.366-368.

Gam, A., Goldstein, L., Karmon, Y., Mintser, I., Grotto, I., Guri, A., Goldberg, A., Ohana, N., Onn, E., Levi, Y. & Bar-Dayana, Y. (2005) 'Comparison of Stress Fractures of Male and Female Recruits during Basic Training in the Israeli Anti-Aircraft Forces', *Military Medicine*, 170 (8), pp.710-712.

Gambling Act 2005. (c.19) London: HMSO.

Gannon, J.R. (1972) 'Stress fractures in the greyhound', *Australian Veterinary Journal*, 48, May, pp.244-250.

Gannon, J. (1987) 'Feeding Greyhounds to Win Races'. In: Clifford, R.J., ed. *The Racing Greyhound: Volume 4*. London: World Greyhound Racing Federation, pp. 97-109.

Genders, R. (1975) *The Greyhound and Greyhound Racing*. London: Sporting Handbooks Ltd.

Genders, R. (1981) *The Encyclopaedia of Greyhound Racing*. 3rd ed. London: Pelham Books Ltd.

Gentleman, R. & Ihaka, R. (2014) *R* (version 3.1.1) [Computer program]. Vienna: The R Foundation for Statistical Computing. Available from: <http://www.r-project.org/index.html>.

Gibbs, C. (1997) 'The BVA/KC scoring scheme for control of hip dysplasia: interpretation of criteria', *Veterinary Record*, 141, pp.275-284.

Giesecke, K., Hamann, H., Stock, K.F., Woehlke, A., Sieme, H. & Distl, O. (2008) 'Evaluation of SPATA1-associated markers for stallion fertility', *Animal Genetics*, 40, pp.359-365.

Giladi, M., Milgrom, C., Kastan, H., Stein, M., Chisin, R. & Dizian, R. (1986) 'Recurrent stress fractures in military recruits. One-year follow-up of 66 recruits', *Journal of Bone & Joint Surgery (British Volume)*, 68 (3), pp.439-441.

Gillette, R. L. (1991) 'The relationship between the track surface and injuries of the racing greyhound', *A report compiled for the Kansas Racing Commission*, pp.1-23.

Gillette, R.L. (1992) 'Track surface influences on the racing greyhound', *Greyhound Review*, 20, pp.41-44.

Gillette, R.L. & Zebas, C. (1991) 'A kinematic and kinetic analysis of the greyhound racing pattern', *A report presented to the Kansas Racing Commision*, pp.15-28.

Gillette, R., Angle, C. & Wakshlag, J. (2006) 'Research design of nutritional studies on the athletic dog needs to take into account the effects of anticipation and conditioning', *Compendium on Continuing Education for the Practicing Veterinary Surgeon*, 28 (4), April, pp.49.

Gillette, R.L., Angle, T.C., Sanders, J.S. & DeGraves, F.J. (2011) 'An evaluation of the physiological effects of anticipation, activity arousal and recovery in sprinting Greyhounds', *Applied Animal Behaviour Science*, 130, pp.101-106.

Gilmour, A.R., Gogel, B.J., Cullis, B.R. & Thompson, R. (2009) *ASREML User Quid*, release 3.

Ginja, M.M.D., Silvestre, A.M., Ferreira, A.J.A., Gonzalo-Orden, J.M., Orden, M.A., Melo-Pinto, P., Llorens-Pena, M.P. & Colaço, J. (2008) 'Passive hip laxity in Estrela Mountain dog - Distraction index, heritability and breeding values', *Acta Veterinaria Hungarica*, 56 (3), pp.303-312.

Ginja, M.M.D., Silvestre, A.M., Gonzalo-Orden, J.M. & Ferreira, A.J.A. (2010) 'Diagnosis, genetic control and preventive management of canine hip dysplasia: A review', *The Veterinary Journal*, 184, pp.269-276.

Givon, U., Friedman, E., Reiner, A., Vered, I., Finestone, A. & Shemer, J. (2000) 'Stress fractures in the Israeli defense forces from 1995 to 1996', *Clinical Orthopaedics & Related Research*, 373, pp.227-232.

Glenk, L.M., Kothgassner, O.D., Stetina, B.U., Palme, R., Kepplinger, B. & Baran, H. (2013) 'Therapy dogs' salivary cortisol levels vary during animal-assisted interventions', *Animal Welfare*, 22, pp.369-378.

Goddard, M.E. & Beilharz, R.G. (1982) 'Genetic and Environmental Factors Affecting the Suitability of Dogs as Guide Dogs for the Blind', *Theoretical and Applied Genetics*, 62, pp.97-102.

Goddard, M.E. & Beilharz, R.G. (1985) 'A Multivariate Analysis of the Genetics of Fearfulness in Potential Guide Dogs', *Behaviour Genetics*, 15 (1), pp.69-89.

Goddard, M.E. & Hayes, B.J. (2009) 'Mapping genes for complex traits in domestic animals and their use in breeding programmes', *Nature Reviews Genetics*, 10, June, pp.381-391.

Gómez, M.D., Valera, M. & Molina, A. (2010a) 'Genetic analysis of racing performance of trotter horses in Spain', *Livestock Science*, 127, pp.197-204.

Gómez, M.D., Menendez-Buxadera, A., Valera, M. & Molina, A. (2010b) 'Estimation of genetic parameters for racing speed at different distances in young and adult Spanish Trotter horses using the random regression model', *Journal of Animal Breeding and Genetics*, 127, pp.385-394.

Gómez, M.D., Varona, L., Molina, A. & Valera, M. (2011) 'Genetic evaluation of racing performance in trotter horses by competitive models', *Livestock Science*, 140, pp.155-160.

Gower, S. & Payne, R. (2010) 'Diseases of racing greyhounds', *Veterinary Record*, 167, pp.226.

Grandjean, D. & Paragon, B.M. (1992) 'Nutrition of racing and working dogs. Part I. Energy metabolism of dogs', *Compendium on Continuing Education for the Practising Veterinarian*, 14, pp.1608-1615.

Greyhound Board of Great Britain. (2009) *Greyhound Board of Great Britain (GBGB)*. [Online] Available from: <http://www.gbgb.org.uk> (Accessed 5 January 2010).

Greyhound Board of Great Britain. (2011) *Guidance for Veterinary Surgeons - Use of norethisterone for oestrus suppression in racing bitches in Great Britain*. [Online]

Available from:

<http://www.gbgb.org.uk/files/Norethisterone%20Guidance%20Notes.pdf>

(Accessed: 11 March 2014).

Greyhound Board of Great Britain. (2014) *Greyhound Board of Great Britain Rules of Racing*. 6th ed. London: Greyhound Board of Great Britain Ltd.

Greyhound Board of Great Britain. (2015) *Annual Report & Accounts 2015*. London: Greyhound Board of Great Britain Ltd.

Greyhound-Data (2014) *Greyhound-Data*. [Online] Available from:

<http://www.greyhound-data.com/> (Accessed 5 March, 2014)

Greyhound-Data (2016) *Greyhound-Data Sire-Pages* [Online] Available from:

<http://www.greyhound-data.com/sirepages.htm?z=pukQED> (Accessed 14 January, 2016)

Greyhound Racing Association Limited (2014) *Abbreviations Used in the Race Card*.

Birmingham: Greyhound Racing Association Ltd.

Greyhound Racing Association of America. (2015) *Greyhound Racing Association of America* [Online] Available from: <http://www.gra-america.org/> (Accessed: 1

January 2016).

Greyhound Racing New South Wales. (2015) *Greyhound Racing New South Wales*.

[Online] Available from: <http://www.grnsw.com.au/> (Accessed 1 January 2016).

Greyhound Racing New Zealand. (2015) *Greyhound Racing New Zealand*. [Online]

Available from: <https://www.thedogs.co.nz/> (Accessed 1 January 2016).

Greyhound Stud Book & National Coursing Club. (2014) *Greyhound Stud Book & National Coursing Club*. [Online] Available from: www.greyhoundstudbook.co.uk (Accessed 8 January 2014).

Guilliard, M.J. (1997) 'Dorsal radiocarpal ligament sprain causing intermittent carpal lameness in high activity dogs', *Journal of Small Animal Practice*, 38, October, pp.463-465.

Guilliard, M.J. (1998) 'Enthesiopathy of the short radial collateral ligaments in racing greyhounds', *Journal of Small Animal Practice*, 39, May, pp.227-230.

Guilliard, M.J. (2000) 'Fractures of the central tarsal bone in eight racing greyhounds', *Veterinary Record*, 147, October, pp.512-515.

Guilliard, M.J. (2001) 'Accessory carpal bone displacement in two dogs', *Journal of Small Animal Practice*, 42, December, pp.603-606.

Guilliard, M.J. (2003a) 'Proximal interphalangeal joint instability in the dog', *Journal of Small Animal Practice*, 44, September, pp.399-403.

Guilliard, M.J. (2003b) 'Dorsal tarsal instability in three racing greyhounds', *Journal of Small Animal Practice*, 44, September, pp.415-417.

Guilliard, M.J. (2005) 'Centrodistal joint lameness in dogs', *Journal of Small Animal Practice*, 46, April, pp.199-202.

Guilliard, M.J. (2010) 'Third tarsal bone fractures in the greyhound', *Journal of Small Animal Practice*, 51, December, pp.635-641.

Guilliard, M.J. (2013) 'Conservative management of fractures of the third metatarsal bone in the racing greyhound', *Journal of Small Animal Practice*, 54, October, pp.507-511.

Guilliard, M.J & Mayo, A.K. (2000a) 'Sprain of the short radial collateral ligament in a racing greyhound', *Journal of Small Animal Practice*, 41, April, pp.169-171.

Guilliard, M.J & Mayo, A.K. (2000b) 'Tears of the palmar superficial fascia in five racing greyhounds and a Labrador retriever', *Journal of Small Animal Practice*, 41, May, pp.218-220.

Guilliard, M.J. & Mayo, A.K. (2001) 'Subluxation/luxation of the second carpal bone in two racing greyhounds and a Staffordshire bull terrier', *Journal of Small Animal Practice*, 42, July, pp.356-359.

Gunn, H.M. (1978) 'The proportions of muscle, bone and fat in two different types of dog', *Research in Veterinary Science*, 24 (3), pp.277-82.

Guo, G., Zhou, Z., Wang, Y., Zhao, K., Zhu, L., Lust, G., Hunter, L., Friedenber, S., Li, J., Zhang, Y., Harris, S., Jones, P., Sandler, J., Krotscheck, U., Todhunter, R. & Zhang, Z. (2011) 'Canine hip dysplasia is predictable by genotyping', *Osteoarthritis and Cartilage*, 19, pp.420-429.

Haberland, A.M., Borstel, U. König von, Simianer, H. & König, S. (2012) 'Integration of genomic information into sport horse breeding programs for optimization of accuracy of selection', *Animal*, 6 (9), pp.1369-1376.

Hardy, R. & Cooper, M.S. (2010) 'Adrenal gland and bone', *Archives of Biochemistry and Biophysics*, 503 (1), pp.137-145.

Hargreaves, A.L. & Hutson, G.D. (1990) 'Changes in heart rate, plasma cortisol and haematocrit of sheep during a shearing procedure', *Applied Animal Behaviour Science*, 26, pp.91-101.

Harris, D.L. & Newman, S. (1994) 'Breeding for profit: synergism between genetic improvement and livestock production (a review)', *Journal of Animal Science*, 72, pp.2178-2200.

Harslof, T., Tofteng, C.L., Husted, L.B., Nyegaard, M., Borglum, A., Carstens, M., Stenkjaer, L., Brixen, K., Eiken, P., Jensen, J-E.B., Mosekilde, L., Rejnmark, L. & Langdahl, B.L. (2011) 'Polymorphisms of the peroxisome proliferator-activated receptor γ (PPAR γ) gene are associated with osteoporosis', *Osteoporosis International*, 22, pp.2655-2666.

Hasler, H., Flury, C., Menet, S., Haase, B., Leeb, T., Simianer, H., Poncet, P.A. & Rieder, S. (2011) 'Genetic diversity in an indigenous horse breed – implications for mating strategies and the control of future inbreeding', *Journal of Animal Breeding and Genetics*, 128, pp.394-406.

Hayashidani, H., Omi, Y., Ogawa, M., & Fukotomi, K. (1988) 'Epidemiological studies on the expectation of life for dogs computed from animal cemetery records', *Japanese Journal of Veterinary Science*, 50, pp.1003–1008.

Heilbrun, A.B. (2004) *Competitiveness in type A College students and stress: examining some untested assumptions*. In: *Disordered and Deviant Behaviour: Learning Gone Awry*. Ed M.D. Lanham. University Press of America. p.99.

Hekman, J.P., Karas, A.Z. & Dreschel, N.A. (2012) 'Salivary cortisol concentrations and behavior in a population of healthy dogs hospitalized for elective procedures', *Applied Animal Behaviour Science*, 141, pp.149-157.

Hellhammer, D.H., Wüst, S. & Kudielka, B.M. (2009) 'Salivary cortisol as a biomarker in stress research', *Psychoneuroendocrinology*, 34, pp.163-171.

Hellsten, E. Thorén, Viklund, Å., Koenen, E.P.C., Ricard, A., Bruns, E. & Philipsson, J. (2006) 'Review of genetic parameters estimated at stallion and young horse performance tests and their correlations with later results in dressage and show-jumping competition', *Livestock Science*, 103, pp.1-12.

Hellsten, E. Thorén, Jorjani, H. & Philipsson, J. (2009) 'Genetic correlations between similar traits in the Danish and Swedish Warmblood sport horse populations', *Livestock Science*, 124, pp.15-20.

Helton, W.S. (2009) 'Exceptional Running Skill in Dogs Requires Extensive Experience', *The Journal of General Psychology*, 136 (3), pp.323-326.

Helton, W.S. (2010) 'Does perceived trainability of dog (*Canis lupus familiaris*) breeds reflect differences in learning or differences in physical ability?' *Behavioural Processes*, 83, pp.315-323.

Henderson, C.R. (1950) 'Estimation of genetic parameters', *The Annals of Mathematical Statistics*, 21, pp.309-310.

Henderson, C.R. (1963) 'Selection index and the expected genetic advance', In: Hanson, W.D & Robinson, H.F. (eds.), *Statistical Genetics and Plant Breeding: A Symposium and Workshop*, pp.141-163. Washington, D.C: National Academies of Sciences, Engineering and Medicine.

Henderson, C.R. (1973) 'Sire evaluation and genetic trends', In: *Proceedings of the animal breeding and genetics symposium in honor of Dr J. L. Lush*, pp.10-41. Champaign, IL: American Society of Animal Science.

Henderson, C.R. (1975) 'Best Linear Unbiased Estimation and Prediction under a Selection Model', *Biometrics*, 31, pp.423-447.

Henderson, C.R. (1976) 'A simple method for the inverse of a numerator relationship matrix used in prediction of breeding values', *Biometrics*, 32, pp.69-83.

Henderson, C.R. (1984) *Applications of linear models in animal breeding*. Guelph, Ontario: University of Guelph.

Hennessey, M.B., Williams, M.T., Miller, D.D., Douglas, C.W. & Voith, V.L. (1998) 'Influence of male and female petters on plasma cortisol and behaviour: can human interaction reduce the stress of dogs in a public shelter?' *Applied Animal Behaviour Science*, 64 (1), pp.63-77.

Hercock, C.A. (2010) *Specialisation for fast locomotion: performance, cost and risk*. PhD Thesis. University of Liverpool.

Hercock, C.A., Innes, J.F., McConnell, F., Guilliard, M.J., Ness, M.G., Hodson, D. & Young, I.S. (2011) 'Observer variation in the evaluation and classification of severe central tarsal bone fractures in racing Greyhounds', *Veterinary and Comparative Orthopaedics and Traumatology*, 3, pp.215-222.

Hewson, C.J. (2003) 'What is animal welfare? Common definitions and their practical consequences', *Canadian Veterinary Journal*, 44, pp.496-499.

Hickman, J. (1975) 'Greyhound injuries', *Journal of Small Animal Practice*, 16, pp.455-460.

Higgins, A. & Nicholas, F.W. (2008) 'The breeding of pedigree dogs: time for strong leadership', *Veterinary Journal*, 178 (2), pp.157-158.

Hill, R.C. (1998) 'The Nutritional Requirements of Exercising Dogs', *The Journal of Nutrition*, 128 (12 Suppl.), December, pp.2686S-2690S.

Hill, R.C., Bloomberg, M.S., Legrand-Defretin, V., Burger, I.H., Hillcock, S.M., Sundstrom, D.A. & Jones, G.L. (2000) 'Maintenance energy requirements and the effect of diet on performance of racing Greyhounds', *American Journal of Veterinary Research*, 61 (12), December, pp.1566-1573.

Hill, R.C., Lewis, D.D., Randell, S.C., Scott, K.C., Omori, M., Jackson, M., Sundstrom, D.A., Jones, G.L., Speakman, J.R., Doyle, C. & Butterwick, R.F. (2001) 'Effect of increased dietary protein and decreased dietary carbohydrate on performance and body in racing Greyhounds', *American Journal of Veterinary Research*, 62 (3), March, pp.440-447.

Hill, R.C., Lewis, D.D., Randell, S.C., Scott, K.C., Omori, M., Sundstrom, D.A., Jones, G.L., Speakman, J.R. & Butterwick, R.F. (2005) 'Effect of mild restriction of food intake on the speed of racing Greyhounds', *American Journal of Veterinary Research*, 66 (6), June, pp.1065-1070.

Hill, E.W., McGivney, B.A. & MacHugh, D.E. (2010a) 'Targets of selection in the Thoroughbred genome contain exercise-relevant gene SNPs associated with elite racecourse performance', *Animal Genetics*, 41 (Suppl. 2), Article Number 552.

Hill, E.W., McGivney, B.A., Gu, J., Whiston, R., MacHugh, D.E. (2010b) 'A genome-wide SNP-association study confirms a sequence variant (g.66493737C>T) in the equine myostatin (MSTN) gene as the most powerful predictor of optimum racing distance for Thoroughbred racehorses', *BMC Genetics*, 11, pp.552-562.

Hinchcliff, K.W., Olson, J., Crusberg, C., Kenyon, J., Long, R., Royle, W., Weber, W., Burr, J. (1993) 'Serum biochemical changes in dogs competing in a long-distance sled race', *Journal of the American Veterinary Medical Association*, 202 (3), February, pp.401-405.

Hopkins, D.L., Fogarty, N.M. & Mortimer, S.I. (2011) 'Genetic related effects on sheep meat quality', *Small Ruminant Research*, 101, pp.160-172.

Horvarth, S.J., Couto, C.G., Kontur, K., Bohenko, L., Iazbik, M.C., Marin, L.M., Hudson, D., Chase, J., Frye, M. & DeNicola, D.B. (2014) 'Effects of racing on reticulocyte concentrations in Greyhounds', *Veterinary Clinical Pathology*, 43 (1), March, pp.15-23.

Hudson, P.E., Corr, S.A. & Wilson, A.M. (2012) 'High speed galloping in the cheetah (*Acinonyx jubatus*) and the racing greyhound (*Canis familiaris*): spatio-temporal and kinetic characteristics', *The Journal of Experimental Biology*, 215 (14), pp.2425-2434.

Huizinga, H.A., Werf, J.H.J. van der, Korver, S. & Meij, G.J.W. van der (1991a) 'Stationary performance testing of stallions from the Dutch Warmblood riding horse population. 1. Estimated genetic parameters of scored traits and the genetic relation with dressage and jumping competition from offspring of breeding stallions', *Livestock Production Science*, 27, pp.231-244.

Huizinga, H.A., Korver, S. & Meij, G.J.W. van der (1991b) 'Stationary Performance testing of stallions from the Dutch Warmblood riding horse population. 2. Estimated heritabilities of and correlations between successive judgements of performance traits', *Livestock Production Science*, 27, pp.245-254.

Hunting Act 2004 (England and Wales). (c.37), London: HMSO.

Huo, Y., Wang, Y., Lust, G., Zhu, L., Zhang, Z. & Todhunter, R.J. (2010) 'Retrospective Analysis for Genetic Improvement of Hip Joints of Cohort Labrador Retrievers in the United States: 1970–2007', *PLOS One*, 5 (2), pp.1-9.

Huson, H.J., Parker, H.G., Runstadler, J. & Ostrander, E.A. (2010) 'A genetic dissection of breed composition and performance enhancement in the Alaskan sled dog', *BMC Genetics*, 11, Article Number 71.

Iddon, J., Lockyer, R.H. & Frean, S.P. (2014) 'The effect of season and track condition on injury rate in racing greyhounds', *Journal of Small Animal Practice*, 55, August, pp.399-404.

Iles, M.M. (2011) 'Chapter 7: Genome-Wide Association Studies'. In: Teare, M.D. (ed.) *Genetic Epidemiology*. London: Humana Press.

Ilkiw, J.E., Davis, P.E. & Church, D.B. (1989) 'Hematologic, biochemical, blood-gas, and acid-base values in greyhounds before and after exercise.', *American Journal of Veterinary Research*, 50 (4), April, pp.583-586.

Illumina, Inc. (2009) *Infinium® HD Assay Ultra Protocol Guide*. Revision B. [Online] San Diego: Illumina, Inc. Available from: http://support.illumina.com/content/dam/illumina-support/documents/myillumina/67f59f89-51ee-44d6-b1bb-a53dcb5bd01e/infinium_hd_ultra_user_guide_11328087_revb.pdf

Illumina, Inc. (2012) *Genomic Selection - A Paradigm Shift in Animal Breeding*. 2012 [Online] Available from: http://res.illumina.com/documents/products/technotes/technote_ag_genomic_selection.pdf (Accessed: 27 September 2013).

Ireland, B.W. (1989) 'Greyhound race track design related to injury', *Proc. No. 122 Greyhound Medicine and Surgery*, Sydney, Australia, September 25-29, pp.355-365.

Irish Coursing Club. (2014) *Irish Coursing Club*. [Online] Available from: <http://www.irishcoursingclub.ie> (Accessed 10 January 2014).

Irish Greyhound Board. (2007) *Irish Greyhound Board Annual Report 2007*. [Online] Available from: <http://www.igb.ie> (Accessed 7 July 2010).

Irish Greyhound Board. (2010) *Irish Coursing Club*. [Online] Available from: <http://www.igb.ie/> (Accessed 7 July 2010).

Islam, M.M., Renwick, A., Lamprinopoulou, C. & Klerkx, L. (2013) 'Innovation in Livestock Genetic Improvement', *EuroChoices*, 12 (1), pp.42-47.

Iwasiow, R.M., James, C.M.P. & Niles, J. (2009) *Quality, Yield, Performance and Bacterial Content of DNA from canine saliva collected and purified using Oragene ANIMAL*. DNA Genotek Technical Bulletin PD-WP-014.

Jelinek, J. (1988) 'Differences in the manifested racing performance of the English Thoroughbred: evaluation of horses of different sexes and years of birth by parametric and non-parametric test', *Scientia Agricola*, 2, pp.131-138.

Jensen, J., Mantysaari, E.A., Madsen, P. & Thompson, R. (1997) 'Residual Maximum Likelihood Estimation of (Co)Variance Components in Multivariate Mixed Linear Models using Average Information', *Journal of the Indian Society of Agricultural Statistics*, 49, pp.215-236.

Johnson, K.A. (1987) 'Accessory Carpal Bone Fractures in the Racing Greyhound Classification and Pathology', *Veterinary Surgery*, 16 (1), pp.60-64.

Johnson, K.A., Piermattei, D.L., Davis, P.E. & Bellenger, C.R. (1988) 'Characteristics of accessory carpal bone fractures in 50 racing greyhounds', *Veterinary & Comparative Orthopaedics & Traumatology*, 2, pp.104-107.

Johnson, E.O., Kamilaris, T.C., Chrousos, G.P. & Gold, P.W. (1992) 'Mechanisms of stress: a dynamic overview of hormonal and behavioural homeostasis', *Neuroscience and Biobehavioral Reviews*, 16, pp.115-130.

Johnson, K.A., Muir, P., Nicoll, R.G. & Roush, J.K. (2000) 'Asymmetric Adaptive Modeling of Central Tarsal Bones in Racing Greyhounds', *Bone*, 27 (2), August, pp.257-263.

Johnson, K.A., Skinner, G.A. & Muir, P. (2001) 'Site-specific adaptive remodeling of Greyhound metacarpal cortical bone subjected to asymmetrical cyclic loading', *American Journal of Veterinary Research*, 62 (5), May, pp.787-793.

Johnson, A.D. (2009) 'Single-Nucleotide Polymorphism Bioinformatics A Comprehensive Review of Resources', *Circulation Cardiovascular Genetics*, 2, pp.530-536.

Jones, B.H., Thacker, S.B, Gilchrist, J., Kimsey, C.D. & Sosin, D.M. (2002) 'Prevention of Lower Extremity Stress Fractures in Athletes and Soldiers: A Systematic Review', *Epidemiologic Reviews*, 24 (2), pp.228-247.

Jones, A.C. & Josephs, R.A. (2006) 'Interspecies hormonal interactions between man and the domestic dog (*Canis familiaris*)', *Hormones and Behavior*, 50, pp.393-400.

Jones, J.M. (2009) 'Repair of hock fractures in racing greyhounds', *Veterinary Record*, 165, October, pp.477-478.

Kaneene, J.B., Mostosky, U.V. & Padgett, G.A. (1997) 'Retrospective cohort study of changes in hip joint phenotype of dogs in the United States', *Journal of the American Veterinary Medical Association*, 211, pp.1542-1544.

Kanis, E., Greef, K.H. de, Hiemstra, A. & Arendonk, J.A.M. van (2005) 'Breeding for societally important traits in pigs', *Journal of Animal Science*, 83, pp.948-957.

Karlsson, E.K., Baranowska, I., Wade, C.M., Hillbertz, N.H.C. Salmon, Zody, M.C., Anderson, N., Biagi, T.M., Patterson, N., Pielberg, G. Rosengren, Kulbokas, E.J., Comstock, K.E., Keller, E.T., Mesirov, J.P., Euler, H. von, Kämpe, O., Hedhammar, A., Lander, E.S., Andersson, G., Andersson, L. & Lindblad-Toh, K. (2007) 'Efficient mapping of mendelian traits in dogs through genome-wide association', *Nature Genetics*, 39 (11), November, pp.1321-1328.

Karlsson, E.K. & Lindblad-Toh, K. (2008) 'Leader of the pack: gene mapping in dogs and other model organisms', *Nature Reviews Genetics*, 9, September, pp.713-725.

Kay, R. & Hall, C. (2009) 'The use of a mirror reduces isolation stress in horses being transported by trailer', *Applied Animal Behaviour Science*, 116, pp.237-243.

Kaygisiz, A. (2013) 'Estimation of genetic parameters and breeding values for dairy cattle using test-day milk yield records', *The Journal of Animal and Plant Sciences*, 23 (2), pp.345-349.

Ke, X., Kennedy, L.J., Short, A.D., Seppälä, E.H., Barnes, A., Clements, D.N., Wood, S.H., Carter, S.D., Happ, G.M., Lohi, H. & Ollier, W.E.R. (2010) 'Assessment of the functionality of genome-wide canine SNP arrays and implications for canine disease association studies', *Animal Genetics*, 42, pp.181-190.

Kearsley, C.G.S., Woolliams, J.A., Coffey, M.P. & Brotherstone, S. (2008) 'Use of competition data for genetic evaluations of eventing horses in Britain: Analysis of the dressage, showjumping and cross country phases of eventing competition', *Livestock Science*, 118, pp.72-81.

Kędzierski, W., Strzelec, K., Cywińska, A. & Kowalik, S. (2013) 'Salivary Cortisol Concentration in Exercised Thoroughbred Horses', *Journal of Equine Veterinary Science*, 33, pp.1106-1109.

Kędzierski, W., Cywińska, A., Strzelec, K. & Kowalik, S. (2014) 'Changes in salivary and plasma cortisol levels in Purebred Arabian horses during race training session', *Animal Science Journal*, 85, pp.313-317.

Keene, R.B. & Yarborough, J.H. (1966) 'Lameness in the greyhound', *Veterinary Scope*, 11: 2.

The Kennel Club. (2014) *The UK Kennel Club*. [Online] Available from: <http://www.thekennelclub.org.uk> (Accessed: 8 January 2014).

Kesl, L.D. & Engen, R.L. (1998) 'Effects of NaHCO₃ loading on acid-base balance, lactate concentration and performance in racing greyhounds', *Journal of Applied Physiology*, 85, pp.1037-1043.

Kiel, D.P., Demissie, S., Dupois, J., Lunetta, K.L., Murabito, J.M. & Karasik, D. (2007) 'Genome-wide association with bone mass and geometry in the Framingham Heart Study', *BMC Medical Genetics*, 8 (Suppl 1):S14.

Kiley-Worthington, M. (1989) 'Ecological, Ethological, and Ethically Sound Environments for Animals: Toward Symbiosis', *Journal of Agricultural Ethics*, 2, pp.323-347.

Kirkness, E.F., Bafna, V., Halpern, A.L., Levy, S., Remington, K., Rusch, D.B., Delcher, A.L., Pop, M., Wang, W., Fraser, C.M. & Venter, J.C. (2003) 'The Dog Genome: Survey Sequencing and Comparative Analysis', *Science*, 301 (5641), pp.1898-1903.

Kiss, Z.S., Khan, K.M. & Fuller, P.J. (1993) 'Stress Fractures of the Tarsal Navicular Bone: CT Findings in 55 Cases', *American Journal of Roentgenology*, 160, January, pp.111-115.

Kobelt, A.J., Hemsworth, P.H., Barnett, J.L. & Butler, K.L. (2003) 'Sources of sampling variation in saliva cortisol in dogs', *Research in Veterinary Science*, 75, pp.157-161.

Koenen, E.P.C., Veldhuizen, A.E. van & Brascamp, E.W. (1995) 'Genetic parameters of linear scored conformation traits and their relation to dressage and show-jumping performance in the Dutch Warmblood Riding Horse population', *Livestock Production Science*, 43, pp.85-94.

Kohnke, J. (2005) *Greyhound Athlete* [Online] Available from:
<http://www.greyhoundracingtoday.com/articles/GREYHOUND-ATHLETE.html>
(Accessed 4 April 2012).

Kohnke, J. (2012) 'Feeding the Racing Greyhound Feeding for Performance', *The Society of Greyhound Veterinarians Conference*, 13 May, Belle Vue Greyhound Stadium, Manchester. [Online] Available from: http://www.greyhoundvets.co.uk/sgv_conference.html (Accessed: 25 February 2014).

Kolevská, J., Brunclík, V. & Svoboda, M. (2003) 'Circadian Rhythm of Cortisol Secretion in Dogs of Different Daily Activities', *Acta Veterinaria Brno*, 72, pp.599-605.

Korpelainen, R., Orava, S., Karpakka, J., Siira, P. & Hulkko, A. (2001) 'Risk Factors for Recurrent Stress Fractures in Athletes', *The American Journal of Sports Medicine*, 29 (3), pp.304-310.

Korvala, J., Hartikka, H., Pihlajamäki, H., Solovieva, S., Ruohola, J.P., Sahi, T., Barral, S., Ott, J., Ala-Kokko, L. & Männikkö, M. (2010) 'Genetic predisposition for femoral neck stress fractures in military conscripts', *BMC Genetics*, 11, Article Number 95.

Kougioumtzis, A., Valergakis, G.E., Oikonomou, G., Arsenos, G. & Banos, G. (2014) 'Profile and genetic parameters of dairy cattle locomotion score and lameness across lactation', *Animal*, 8 (1), pp.20-27.

Kowal, D.M. (1980) 'Nature and causes of injuries in women resulting from an endurance training program', *The American Journal of Sports Medicine*, 8 (4), pp.265-269.

Koyama, T., Omata, Y. & Saito, A. (2003) 'Changes in salivary cortisol concentrations during a 24-hour period in dogs', *Hormone and Metabolic Research*, 35 (6), pp.355-357.

Langlois, B., Blouin, C. & Tavernier, A. (1996) 'Nouveaux résultats d'estimation de l'héritabilité des gains en des pur-sang en France' [New results on the estimation of the heritabilities of earnings of thoroughbreds in France], *Genetics Selection Evolution*, 28, pp.275-283. (In French).

Langlois, B. & Vrijenhoek, T. (2004) 'Qualification status and estimation of breeding value in French trotters', *Livestock Production Science*, 89, pp.187-194.

Langlois, B. & Blouin, C. (2004) 'Practical efficiency of breeding value estimations based on annual earnings of horses for jumping, trotting, and galloping races in France', *Livestock Production Science*, 87, pp.99-107.

LaVecchio, D., Marin, L.M., Baumwart, R., Iazbik, M.C., Westendorf, N. & Couto, C.G. (2009) 'Serum Cardiac Troponin I Concentration in Retired Racing Greyhounds', *Journal of Veterinary Internal Medicine*, 23, pp.87-90.

Lavrijsen, I.C.M., Leegwater, P.A.J., Martin, A.J., Harris, S.J., Tryfonidou, M.A., Heuven, H.C.M. & Hazewinkel, H.A.W. (2014) 'Genome Wide Analysis Indicates Genes for Basement Membrane and Cartilage Matrix Proteins as Candidates for Hip Dysplasia in Labrador Retrievers', *PLOS One*, 9 (1), January, pp.1-7.

Lavrijsen, I.C.M., Leegwater, P.A.J., Wangdee, C., Steenbeek, F.G. van, Schwencke, M., Breur, G.J., Meutstege, F.J., Nijman, I.J., Cuppen, E., Heuven, H.C.M. & Hazewinkel, H.A.W. (2014) 'Genome-wide survey indicates involvement of loci on canine chromosomes 7 and 31 in patellar luxation in flat-coated retrievers', *BMC Genetics*, 15, Article Number 64.

Leadon, D.P. & Mullins, E. (1991) 'Relationship between kennel size and stress in greyhounds transported short distances by air', *Veterinary Record*, 129, pp.70-73.

Lee, S.J. (2007) 'Sprinting without myostatin: a genetic determinant of athletic prowess', *Trends in Genetics*, 23 (10), pp.475-477.

Leighton, E.A. & Booker, J.H. (2003) 'How To Use Estimated Breeding Values to Genetically Improve Dog Guides', *Presented at a Meeting of the "Original Group"*, 11-12 September, The Seeing Eye, Inc., New Jersey. [Online] Available from: <http://www.seeingeye.org/cms/uploads/howtouseebv.pdf> (Accessed: 27 September 2013).

Lennox, A. (1987) *Greyhounds - The Sporting Breed*. London: The Sportsman's Press.

Leppänen, M. & Saloniemi, H. (1999) 'Controlling canine hip dysplasia in Finland', *Preventive Veterinary Medicine*, 42, pp.121-131.

Leppänen, M., Mäki, K., Juga, J. & Saloniemi, H. (2000) 'Estimation of heritability for hip dysplasia in German Shepherd Dogs in Finland', *Journal of Animal Breeding and Genetics*, 117, pp.97-103.

Lequarré, A.S., Andersson, L., André, C., Fredholm, M., Hitte, C., Leeb, T., Lohi, H., Lindblad-Toh, K. & Georges, M. (2011) 'LUPA: A European initiative taking advantage of the canine genome architecture for unravelling complex disorders in both human and dogs', *The Veterinary Journal*, 189, pp.155-159.

Leroy, G. & Abitbol, M. (2010) *Breeding Strategies Against Genetic Disorders in Dog Breeds* [Online] Available from: <http://www.kongressband.de/wcgalp2010/assets/pdf/0319.pdf> (Accessed: 27 September 2013).

Lewinski, M. von, Biau, S., Erber, R., Ille, N., Aurich, J., Faure, J.M., Möstl, E. & Aurich, C. (2013) 'Cortisol release, heart rate and heart rate variability in the horse and its rider: Different responses to training and performance', *The Veterinary Journal*, 197, pp.229-232.

Lewis, T.W., Woolliams, J.A. & Blott, S.C. (2010a) 'Optimisation of breeding strategies to reduce the prevalence of inherited disease in pedigree dogs', *Animal Welfare*, 19 (S), pp.93-98.

Lewis, T.W., Blott, S.C. & Woolliams, J.A. (2010b) 'Genetic Evaluation of Hip Score in UK Labrador Retrievers', *PLOS One*, 5 (10), October, pp.1-10.

Lewis, T.W., Woolliams, J.A. & Blott, S.C. (2010c) 'Genetic Evaluation of the Nine Component Features of Hip Score in UK Labrador Retrievers', *PLOS One*, 5 (10), p.1-8.

Lewis, T., Swift, S., Woolliams, J.A. & Blott, S. (2011) 'Heritability of premature mitral valve disease in Cavalier King Charles spaniels', *The Veterinary Journal*, 188, pp.73-76.

Lewis, T.W., Blott, S.C. & Woolliams, J.A. (2013) 'Comparative analyses of genetic trends and prospects for selection against hip and elbow dysplasia in 15 UK dog breeds', *BMC Genetics*, 14, Article Number 16.

Ley, J.M., Bennett, P.C. & Coleman, G.J. (2009) 'A refinement and validation of the Monash Canine Personality Questionnaire (MCPQ)', *Applied Animal Behaviour Science*, 116, pp.220-227.

Liinamo, A.-E., Berg, L. van den, Leegwater, P.A.J., Schilder, M.B.H., Arendonk, J.A.M. van & Oost, B.A. van. (2007) 'Genetic variation in aggression-related traits in Golden Retriever dogs', *Applied Animal Behaviour Science*, 104, pp.95-106.

Lindberg, S., Strandberg, E. & Swenson, L. (2004) 'Genetic analysis of hunting behaviour in Swedish Flatcoated Retrievers', *Applied Animal Behaviour Science*, 88, pp.289-298.

Lindblad-Toh, K., Wade, C.M., Mikkelsen, T.J., Karlsson, E.K., Jaffe, D.B., Kamal, M., Clamp, M., Chang, J.L., Kulbokas, E.J., Zody, M.C., Mauceli, E., Xie, X., Breen, M., Wayne, R.K., Ostrander, E.A., Ponting, C.P., Galibert, F., Smith, D.R., deJong, P.J., Kirkness, E., Alvarez, P., Biagi, T., Brockman, W., Buler, J., Chin, C.W., Cook, A., Cuff, J., Daly, M.J., DeCaprio, D., Gnerre, S., Grabherr, M., Kellis, M., Kleber, M., Bardeleben, C., Goodstadt, L., Heger, A., Sutter, N.B., Thomas, R., Webber, C. & Lander, E.S. (2005) 'Genome sequence, comparative analysis and haplotype structure of the domestic dog', *Nature*, 438 (8), December, pp.803-819.

Lindblad-Toh, K. (2012) 'Chapter 12: Canine Genomics'. In: Ostrander, E.A. & Ruvinsky, A. (eds.) *The Genetics of the Dog*. 2nd ed. Oxfordshire: CABI Publishing.

Lipscomb, V.J., Lawes, T.J., Goodship, A.E. & Muir, P. (2001) 'Asymmetric densitometric and mechanical adaptation of the left fifth metacarpal bone in racing greyhounds', *Veterinary Record*, 148, March, pp.308-311.

Luger, A., Deuster, P.A. & Kyle, S.B. *et al.* (1987) 'Acute hypothalamic-pituitary-adrenal responses to the stress of treadmill exercise', *The New England Journal of Medicine*, 316, pp.1309-1315.

Lynch, M. & Walsh, B. (1998) *Genetics and Analysis of Quantitative Traits*. Sunderland, MA, USA: Sinauer Associates.

Macfarlane, J.M., Matheson, S.M. & Dwyer, C.M. (2010) 'Genetic parameters for birth difficulty, lamb vigour and lamb sucking ability in Suffolk sheep', *Animal Welfare*, 19 (S), pp.99-105.

Machiela, M.J. & Chanock, S.J. (2014) 'GWAS is going to the dogs', *Genome Biology*, 15, Article Number 105.

Mackinnon, M.C., Bonder, D., Boston, R.C. & Ross, M.W. (2014) 'Analysis of stress fractures associated with lameness in Thoroughbred flat racehorses training on different track surfaces undergoing nuclear scintigraphic examination', *Equine Veterinary Journal*. In Press. Available from: onlinelibrary.wiley.com.ezproxy.liv.ac.uk (Accessed: 01 August 2014).

Madden, R. (2010) 'Imagining the greyhound: 'Racing' and 'rescue' narratives in a human and dog relationship', *Continuum: Journal of Media & Cultural Studies*, 24 (4), August, pp.503-515.

Madsen, P. & Jensen, J. (2013) *DMU package* (version 6, release 5.2) [Computer program]. Aarhus: University of Aarhus.

Magness, S., Ambegaonkar, J.P., Jones, M.T. & Caswell, S.V. (2011) 'Lower Extremity Stress Fracture in Runners: Risk Factors and Prevention', *International Journal of Athletic Therapy & Training*, 16 (4), July, pp.11-15.

Mäki, K., Groen, A.F., Liinamo, A.-E. & Ojala, M. (2002) 'Genetic variances, trends and mode of inheritance for hip and elbow dysplasia in Finnish dog populations', *Animal Science*, 75, pp.197-207.

Malm, S., Fikse, W.F., Danell, B. & Strandberg, E. (2008) 'Genetic variation and genetic trends in hip and elbow dysplasia in Swedish Rottweiler and Bernese Mountain Dog', *Journal of Animal Breeding and Genetics*, 125, pp.403-412.

Marino, C.L., Cober, R.E., Iazbik, M.C. & Couto, C.G. (2011) 'White-Coat Effect on Systemic Blood Pressure in Retired Racing Greyhounds', *Journal of Veterinary Internal Medicine*, 25, pp.861-865.

Marshall, R.J., Scott, K.C., Hill, R.C., Lewis, D.D., Sundstrom, D., Jones, G.L. & Harper, J. (2002) 'Supplemental Vitamin C Appears to Slow Racing Greyhounds', *The Journal of Nutrition*, 132 (6 Suppl. 1), pp.1616S-1621S.

Martig, S., Chen, W., Lee, P.V.S. & Whitton, R.C. (2014) 'Analysis of stress fractures associated with lameness in Thoroughbred flat racehorses training on different track surfaces undergoing nuclear scintigraphic examination', *Equine Veterinary Journal*, 46, pp.408-415.

Massey, J., Short, A.D., Catchpole, B., House, A., Day, M.J., Lohi, H., Ollier, W.E.R. & Kennedy, L.J. (2014) 'Genetics of canine anal furunculosis in the German shepherd dog', *Immunogenetics*, 66, pp.311-324.

Mastorakos, G., Pavlatou, M., Diamanti-Kandarakis, E. & Chrousos, G.P. (2005) 'Exercise and the stress system', *Hormones*, 4, pp.73-89.

McClung, J.P. & Karl, J.P. (2010) 'Vitamin D and stress fracture: the contribution of vitamin D receptor gene polymorphisms', *Nutrition Reviews*, 68 (6), pp.365-369.

McGowan, C.M., Fordham, T. & Christley, R.M. (2002) 'Incidence and risk factors for exertional rhabdomyolysis in thoroughbred racehorses in the United Kingdom', *Veterinary Record*, 151, pp.623-626.

Medica, P., Giacoppo, E., Fazio, E., Pellizzotto, R. & Ferlazzo, A. (2010) 'Cortisol and haematochemical variables of horses during a two day trekking event: effects of preliminary transport', *Equine Veterinary Journal*, 42 (Suppl. 38), pp.167-170.

Meensel, A.S. van & Peers, K. (2010) 'Navicular stress fracture in high-performing twin brothers: A case report', *Acta Orthopaedica Belgica*, 76 (3), pp.407-412.

Meissen, G. (1997) *Populationgenetische Analysen zur Vererbung von Größe und Rennleistung beim Whippet*. PhD thesis. TG-Verlag, Gießen.

Menor-Campos, D.J, Molleda-Carbonell, J.M. & López-Rodríguez, R. (2011) 'Effects of exercise and human contact on animal welfare in a dog shelter', *Veterinary Record*, 169 (15), pp.388-391.

Merck Veterinary Manual (2016) *Tricuspid Valve Dysplasia* [Online]. Available from: <http://www.msdsvetmanual.com/circulatory-system/congenital-and-inherited-anomalies-of-the-cardiovascular-system/tricuspid-valve-dysplasia> (Accessed 5 January 2016).

Merkel, D., Moran, D.S., Yanovich, R., Evans, R.K., Finestone, A.S., Constantini, N. & Israeli, E. (2008) 'The Association between Hematological and Inflammatory Factors and Stress Fractures among Female Military Recruits', *Official Journal of the American College of Sports Medicine*, 40 (11), November, pp.S692-S697.

Meyer, F., Schawalter, P., Gaillard, C. & Dolf, G. (2012) 'Estimation of genetic parameters for behavior based on results of German Shepherd Dogs in Switzerland', *Applied Animal Behaviour Science*, 140, pp.53-61.

The Microchipping of Dogs (England) Regulations 2015. SI 2015/108. London: The Stationary Office Ltd.

Microsoft Corporation (2007) *Access 2007* [Computer program]. Microsoft Corporation.

Microsoft Corporation (2007) *Office Excel 2007* [Computer program]. Microsoft Corporation.

Milgrom, C., Giladi, M., Stein, M., Kashtan, H., Margulies, J.Y., Chisin, R., Steinberg, R. & Aharonson, Z. (1985) 'Stress fractures in military recruits. A prospective study showing an unusually high incidence', *Journal of Bone & Joint Surgery (British Volume)*, 67 (5), pp.732-735.

Moberg, G.P. (2000) *Biological Response to Stress: Implications for Animal Welfare*. In: *The Biology of Animal Stress: Basic Principles and Implications for Animal Welfare*. Eds G.P. Moberg & J.A. Mench. CABI Publishing.

Molyneux, J. (2005) 'Vets on track: working as a greyhound vet', *In Practice*, 27, pp.277-279.

Mori, S. & Burr, D.B. (1993) 'Increased Intracortical Remodeling Following Fatigue Damage', *Bone*, 14, pp.103-109.

Morris, D. (2009) *Training and Racing the Greyhound*. Wiltshire: The Crowood Press Ltd.

Morris, C. & Holloway, L. (2014) 'Genetics and livestock breeding in the UK: Co-constructing technologies and heterogeneous biosocial collectivities', *Journal of Rural Studies*, 33, pp.15-160.

Mortimer, S.I., Werf, J.H.J. van der, Jacob, R.H., Hopkins, D.L., Pannier, L., Pearce, K.L., Gardner, G.E., Warner, R.D., Geesink, G.H., Edwards, J.E. Hocking, Ponnampalam, E.N., Ball, A.J., Gilmour, A.R. & Pethick, D.W. (2014) 'Genetic parameters for meat quality traits of Australian lamb meat', *Meat Science*, 96, pp.1016-1024.

Mosher, D.S., Quignon, P., Bustamante, C.D., Sutter, N.B., Mellersh, C.S., Parker, H.G. & Ostrander, E.A. (2007) 'A Mutation in the Myostatin Gene Increases Muscle Mass and Enhances Racing Performance in Heterozygote Dogs', *PLOS Genetics*, 3 (5), May, pp.0779-0786.

Matteri, R.L., Carroll, J.A. & Dyer, C.J. (2002) *Neuroendocrine responses to stress*. In: The biology of animal stress. Eds: G.P. Moberg, J.A. Mench. CABI Publishing. pp.43-76.

Möstl, E. & Palme, R. (2002) 'Hormones as indicators of stress', *Domestic Animal Endocrinology*, 23, pp.67-74.

Mota, M.D.S., Oliviera, H.N. & Silva, R.G. (1998) 'Genetic and environmental factors that affect the best time of Thoroughbred horses in Brazil', *Journal of Animal Breeding and Genetics*, 115, pp.123-129.

Mota, M.D.S., Abrahão, A.R. & Oliviera, H.N. (2005) 'Genetic and environmental parameters for racing time at different distances in Brazilian Thoroughbreds', *Journal of Animal Breeding and Genetics*, 122, pp.393-399.

Mota, M.D.S. da (2006) 'Genetic correlations between performance at different racing distances in Thoroughbreds', *Livestock Science*, 104, pp.227-232.

Muir, P., Johnson, K.A. & Ruaux-Mason, C.P. (1999) 'In Vivo Matrix Microdamage in a Naturally Occurring Canine Fatigue Fracture', *Bone*, 25 (5), November, pp.571-576.

Nakajima, H., Miyuki, I., Morikawa, Y., Komori, T., Fukuchi, Y., Shibata, F., Okamoto, S. & Kitamura, T. (2009) 'Wnt modulators, SFRP-1, and SFRP-2 are expressed in osteoblasts and differentially regulate hematopoietic stem cells', *Biochemical and Biophysical Research Communications*, 390, pp.65-70.

Nature Education (2014) *Scitable by Nature Education: Glossary Definition* [Online]. Available from: <http://www.nature.com/scitable/definition/population-bottleneck-300> (Accessed 2 June 2015).

Nguyen, T.V. & Eisman, J.A. (2000) 'Genetics of Fracture: Challenges and Opportunities', *Journal of Bone and Mineral Research*, 15 (7), pp.1253-1256.

Nicholas, F.W. (2003) *Introduction to Veterinary Genetics*. 2nd ed. Oxford: Blackwell Publishing Ltd.

Nicholas, F.W. & Wade, C.M. (2011) 'Canine Genetics: A Very Special Issue', *The Veterinary Journal*, 189, pp.123-125.

Nold, J.L., Peterson, L.J. & Fedde, M.R. (1991) 'Physiological changes in the running greyhound (*Canis domesticus*): Influence of race length', *Comparative Biochemistry and Physiology*, 100A (3), pp.623-627.

Oberbauer, A.M., Bell, J.S., Belanger, J.M. & Famula, T.R. (2006) 'Genetic evaluation of Addison's disease in the Portuguese Water Dog', *BMC Veterinary Research*, 2.

Oki, H., Willham, R.L & Sasaki, Y. (1994) 'Genetics of racing performance in the Japanese Thoroughbred horse: I. Description of the data', *Journal of Animal Breeding and Genetics*, 111, pp.121-127.

Oki, H., Sasaki, Y. & Willham, R.L. (1995) 'Genetic parameter estimates for racing time by restricted maximum likelihood in the Thoroughbred horse of Japan', *Journal of Animal Breeding and Genetics*, 112, pp.146-150.

Oki, H., Sasaki, Y. & Willham, R.L. (1997) 'Estimation of genetic correlations between racing times recorded at different racing distances by restricted maximum likelihood in Thoroughbred racehorses', *Journal of Animal Breeding and Genetics*, 114, pp.185-189.

Oliehoek, P.A., Bijma, P. & Meijden, A. van der (2009) 'History and structure of the closed pedigreed population of Icelandic Sheepdogs', *Genetics Selection Evolution*, 41, Article Number 39.

Olsen, H.F., Klemetsdal, G., Ødegård, J. & Árnason, T. (2012) 'Validation of alternative models in genetic evaluation of racing performance in North Swedish and Norwegian cold-blooded trotters', *Journal of Animal Breeding and Genetics*, 129, pp.164-170.

Olsson, E., Näsholm, A., Strandberg, E. & Philipsson, J. (2008) 'Use of field records and competition results in genetic evaluation of station performance tested Swedish Warmblood stallions', *Livestock Science*, 117, pp.287-297.

O'Neill, D.G., Church, D.B., McGreevy, P.D., Thomson, P.C. & Brodbelt, D.C. (2013) 'Longevity and mortality of owned dogs in England', *The Veterinary Journal*, 198, pp.638-643.

Ost, P.C., Dee, J.F., Dee, L.G. & Hohn, R.B. (1987) 'Fractures of the Calcaneus in Racing Greyhounds', *Veterinary Surgery*, 16 (1), pp.53-59.

Ostrander, E.A. & Comstock, K.E. (2004) 'The domestic dog genome', *Current Biology*, 14 (3), February, pp.R98-R99.

Ostrander, E.A. & Wayne, R.K. (2005) 'The canine genome', *Genome Research*, 15, pp.1706-1716.

Ostrander, E.A. & Ruvinsky, A. (eds.) (2012) *The Genetics of the Dog*. 2nd ed. Oxfordshire: CABI Publishing.

O'Sullivan, C.B & Lumsden, J.M. (2002) 'Distal third metacarpal bone palmar cortical stress fractures in four Thoroughbred racehorses', *Equine Veterinary Education*, 14 (2), pp.70-76.

O'Sullivan, C.B & Lumsden, J.M. (2003) 'Stress fractures of the tibia and humerus in Thoroughbred racehorses: 99 cases (1992–2000)', *Journal of the American Veterinary Medical Association*, 222 (4), February, pp.491-498.

Ottenheimer Carrier, L., Cyr, A., Anderson, R.E. & Walsh, C.J. (2013) 'Exploring the dog park: Relationships between social behaviours, personality and cortisol in companion dogs', *Applied Animal Behaviour Science*, 146, pp.96-106.

Ouschan, C., Kuchar, A. & Möstl, E. (2013) 'Measurement of cortisol in dog hair: a noninvasive tool for the diagnosis of hypercortisolism', *Veterinary Dermatology*, 24, pp.428-e94.

Oyama, D., Hyodo, M., Doi, H., Kurachi, T., Takata, M., Koyama, S., Satoh, T. & Watanabe, G. (2014) 'Saliva collection by using filter paper for measuring cortisol levels in dogs', *Domestic Animal Endocrinology*, 46, pp.20-25.

Panigrahi, M. & Parida, S. (2012) 'Genomic selection - Revolutionary breeding practice in Domestic animals', *Veterinary World*, 5 (7), pp.433-436.

Park, K.D. (2011) 'Genetic parameters of finish time in Korean Thoroughbred racehorses', *Livestock Science*, 140, pp.49-54.

Parker, H.G., Kim, L.V., Sutter, N.B., Carlson, S., Lorentzen, T.D., Malek, T.B., Johnson, G.S., DeFrance, H.B., Ostrander, E.A. & Kruglyak, L. (2004) 'Genetic Structure of the Purebred Domestic Dog', *Science*, 304, May, pp.1160-1164.

Parker, H.G. (2012) 'Chapter 3: The History and Relationships of Dog Breeds'. In: Ostrander, E.A. & Ruvinsky, A. (eds.) *The Genetics of the Dog*. 2nd ed. Oxfordshire: CABI Publishing.

Parker, H.G. & Ostrander, E.A. (2005) 'Canine Genomics and Genetics: Running with the Pack', *PLOS Genetics*, 1 (5), November, pp.0507-0513.

Parker, H.G., Shearin, A.L. & Ostrander, E.A. (2010) 'Man's Best Friend Becomes Biology's Best in Show: Genome Analyses in the Domestic Dog', *Annual Review of Genetics*, 44, pp.309-336.

Parkin, T.D.H. (2008) 'Epidemiology of Racetrack Injuries in Racehorses', *Veterinary Clinics Equine Practice*, 24, pp.1-19.

Pastore, C., Pirrone, F., Balzarotti, F., Faustini, M., Pierantoni, L. & Albertini, M. (2011) 'Evaluation of physiological and behavioral stress-dependent parameters in agility dogs', *Journal of Veterinary Behavior*, 6, pp.188-194.

Payne, R.M. (2013a) 'The effect of dioestrus on the racing performance of Greyhounds', *The Veterinary Journal*, 197, pp.670-674.

Payne, R.M. (2013b) 'The effect of spaying on the racing performance of female greyhounds', *The Veterinary Journal*, 198, pp.372-375.

Pech, C.I.V. Manzanilla, Veerkamp, R.F., Calus, M.P.L., Zom, R., Knegsel, A. van, Pryce, J.E. & Haas, Y. de (2014) 'Genetic parameters across lactation for feed intake, fat- and protein-corrected milk, and liveweight in first-parity Holstein cattle', *Journal of Dairy Science*, In Press. Available from: www.sciencedirect.com.ezproxy.liv.ac.uk/science/article/eii/S0022030214004974# (Accessed: 15 August 2014).

Pederson, N.C., Liu, H., Greenfield, D.L. & Echols, L.G. (2012) 'Multiple autoimmune diseases syndrome in Italian Greyhounds: Preliminary studies of genome-wide diversity and possible associations within the dog leukocyte antigen (DLA) complex', *Veterinary Immunology and Immunopathology*, 145, pp.264-276.

Perago, R., Proverbio, D. & Spada, E. (2013) 'Increases in heart rate and serum cortisol concentrations in healthy dogs are positively correlated with an indoor waiting-room environment', *Veterinary Clinical Pathology*, 43 (1), pp.67-71.

Phillips, I.R. (1979) 'A survey of bone fractures in the dog and cat', *Journal of Small Animal Practice*, 20 (11), pp.661-674.

Piercy, R.J., Hinchcliff, K.W., Morley, P.S., DiSilvestro, R.A., Reinhart, G.A., Nelson, S.L., Schmidt, K.E. & Craig, A. Morrie (2001) 'Vitamin E and exertional rhabdomyolysis during endurance sled dog racing', *Neuromuscular Disorders*, 11, pp.278-286.

Piras, A. (2005) 'Customized single hook plate for fractures of metacarpal/tarsal bones in racing greyhounds', *Dialogue*, 2, pp.31-33.

Piras, A. (2008) *Stress fractures in racing greyhounds* [Online] Northern Ireland: The Australian & New Zealand College of Veterinary Scientists. Available from: http://surgery.anzcvs.org.au/surgery_assets/science%20week%202008/stress%20fractures%20in%20racing%20greyhounds.pdf (Accessed: 22 July 2011).

Posta, J., Komlósi, I. & Mihók, S. (2009) 'Breeding value estimation in the Hungarian Sport Horse population', *The Veterinary Journal*, 181, pp.19-23.

Posta, J., Malovhr, S., Mihók, S. & Komlósi, I. (2010) 'Random regression model estimation of genetic parameters for show-jumping results of Hungarian Sporthorses', *Journal of Animal Breeding and Genetics*, 127, pp.280-288.

Pottinger, T.G. (2000) *Genetic Selection to Reduce Stress in Animals*. In: *The Biology of Animal Stress: Basic Principles and Implications for Animal Welfare*. Eds G.P. Moberg & J.A. Mench. CABI Publishing.

Poulter, D.A.L. (1981) 'Injuries to racing greyhounds', *The Veterinary Record*, 109, pp.106.

Příbyl, J., Madsen, P., Bauer, J., Příbylová, J., Šimečková, M., Vostrý, L. & Zavadilová, L. (2013) 'Contribution of domestic production records, Interbull estimated breeding values, and single nucleotide polymorphism genetic markers to the single-step genomic evaluation of milk production', *Journal of Dairy Science*, 96 (3), pp.1865-1873.

Pritchard, T., Coffey, M., Mrode, R. & Wall, E. (2013a) 'Understanding the genetics of survival in dairy cows', *Journal of Dairy Science*, 96 (5), pp.3296-3309.

Pritchard, T., Coffey, M., Mrode, R. & Wall, E. (2013b) 'Genetic parameters for production, health, fertility and longevity traits in dairy cows', *Animal*, 7 (1), pp.34-46.

Prole, J.H.B (1973) 'Some observations on the physiology of reproduction in the Greyhound bitch', *Journal of Small Animal Practice*, 14, pp.781-784.

Prole, J.H.B. (1976) 'A survey of racing injuries in the Greyhound', *Journal of Small Animal Practice*, 17, pp.207-218.

Protection of Wild Mammals (Scotland) Act 2002. (asp.6), Edinburgh: HMSO.

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A.R., Bender, D., Maller, J., Sklar, P., Bakker, P.I.W. de, Daly, M.J. & Sham, P.C. (2007) 'PLINK: A Tool Set for Whole-Genome Association and Population-Based Linkage Analyses', *The American Journal of Human Genetics*, 81, pp.559-575.

Purcell, S. (2009) *PLINK* (version 1.07) [Computer program]. Available from: <http://pngu.mgh.harvard.edu/purcell/plink/>.

Qin, A., Cheng, T.S., Pavlos, N.J., Lin, Z., Dai, K.R. & Zheng, M.H. (2012) 'V-ATPases in osteoclasts: Structure, function and potential inhibitors of bone resorption', *International Journal of Biochemistry & Cell Biology*, 44, pp.1422-1435.

The R Foundation for Statistical Computing. (2014) *R* (version 3.1.1) [Computer program]. Available from: <https://www.r-project.org/>.

Raynaert, R., De Paepe, M. & Peeters, G. (1976) 'Influence of stress, age and sex on serum growth hormone and free fatty acids in cattle', *Hormone & Metabolic Research*, 8, pp.109-114.

Reece, W.O., Erickson, H.H., Goff, J.P. & Uemura (2015) *Chapter 41: Exercise Physiology of Terrestrial Animals*. In: *Duke's Physiology of Domestic Animals*. 13th ed. Wiley-Blackwell.

Reich, D.E., Cargill, M., Bolk, S., Ireland, J., Sabeti, P.C., Richter, D.J., Lavery, T., Kouyoumjian, R., Farhadian, S.F., Ward, R. & Lander, E.S. (2001) 'Linkage disequilibrium in the human genome', *Nature*, 411, May, pp.199-204.

Reimers, T.J., Lawler, D.F., Sutaria, P.M., Correa, M.T. & Erb, H.N. (1990) 'Effects of age, sex and body size on serum concentrations of thyroid and adrenocortical hormones in dogs', *American Journal of Veterinary Research*, 51, pp.454-457.

Retired Greyhound Trust (RGT). (2011) *Retired Greyhound Trust*. [Online] Available from: <https://www.retiredgreyhounds.co.uk/> (Accessed 15 January, 2011).

Ricard, A., Bruns, E. & Cunningham, E.P. (2000) 'Genetics of Performance Traits'. In: Bowling, A.T. & Ruvinsky, A. (eds.) *Genetics of the Horse*. Oxfordshire: CABI Publishing, pp.411-438.

Ricard, A. & Blouin, C. (2011) 'Genetic analysis of the longevity of French sport horses in jumping competition', *Journal of Animal Science*, 89, pp.2988-2994.

Ricard, A. & Chanu, I. (2001) 'Genetic parameters of eventing horse competition in France', *Genetics Selection Evolution*, 33, pp.175-190.

Riggs, C.M. (2002) 'Fractures - A Preventable Hazard of Racing Thoroughbreds?', *The Veterinary Journal*, 163, pp.19-29.

Robson, P.J., Aldston, T.D. & Myburgh, K.H. (2003) 'Prolonged suppression of the innate immune system in the horse following an 80 km endurance race', *Equine Veterinary Journal*, 35 (2), pp.133-137.

Rooney, N.J. & Bradshaw, J.W.S. (2004) 'Breed and sex differences in the behavioural attributes of specialist search dogs - a questionnaire survey of trainers and handlers', *Applied Animal Behaviour Science*, 86, pp.123-135.

Rooney, N.J. & Sargan, D. (2008) *Pedigree dog breeding in the UK: a major welfare concern?* An independent scientific report commissioned by the Royal Society for the Prevention of Cruelty to Animals.

Rose, R.J. & Bloomberg, M.S. (1989) 'Responses to sprint exercise in the greyhound: effects on haematology, serum biochemistry and muscle metabolites', *Research in Veterinary Science*, 47 (2), September, pp.212-218.

Ross, R.A. & Allsopp, A. (2002) 'Stress Fractures in Royal Marine Recruits', *Military Medicine*, 167 (7), July, pp.560-565.

Roughsedge, T., Amer, P.R., Thompson, R. & Slimm, G. (2005) 'Genetic parameters for a maternal breeding goal in beef production', *Journal of Animal Science*, 83, pp.2319-2329.

Rovira, S., Muñoz, A. & Benito, M. (2007) 'Hematologic and biochemical changes during canine agility competitions', *Veterinary Clinical Pathology*, 36 (1), pp.30-35.

Roy, R. & Dee, J. (1994) *Management of articular fractures, sprains and strains*. In: Manual of Small Animal Arthrology, Eds J.E.F. Houlton & R.W. Collinson. British Small Animal Veterinary Association, Cheltenham, pp.55-61.

Royal, M.D., Flint, A.P.F. & Woolliams, J.A. (2002a) 'Genetic and Phenotypic Relationships Among Endocrine and Traditional Fertility Traits and Production Traits in Holstein-Friesian Dairy Cows', *Journal of Dairy Science*, 85 (4), pp.958-967.

Royal, M.D., Pryce, J.E., Woolliams, J.A. & Flint, A.P.F. (2002b) 'The Genetic Relationship between Commencement of Luteal Activity and Calving Interval, Body Condition Score, Production, and Linear Type Traits in Holstein-Friesian Dairy Cattle', *Journal of Dairy Science*, 85 (11), pp.3071-3080.

Ruefenacht, S., Gebhardt-Henrich, S., Miyake, T. & Gaillard, C. (2002) 'A behaviour test on German Shepherd dogs: heritability of seven different traits', *Applied Animal Behaviour Science*, 79, pp.113-132.

Rutherford, S. & Ness, M.G. (2012) 'Dorsal Slab Fracture of the Fourth Carpal Bone in a Racing Greyhound', *Veterinary Surgery*, 41, pp.944-947.

Ruohola, J.P., Laaksi, I., Ylikomi, T., Haataja, R., Mattila, V.M., Sahi, T., Tuohimaa, P. & Pihlajamäki, H. (2006) 'Association Between Serum 25(OH)D Concentrations and Bone Stress Fractures in Finnish Young Men', *Journal of Bone and Mineral Research*, 21 (9), pp.1483-1488.

Rustin, M., Janssens, S., Buys, N. & Gengler, N. (2009) 'Multi-trait animal model estimation of genetic parameters for linear type and gait traits in the Belgian warmblood horse', *Journal of Animal Breeding and Genetics*, 126, pp.378-386.

Ryan, J.E. (1975) *The Inheritance of track performance in Greyhounds*. MSc thesis. Trinity College Dublin.

Saastamoinen, M.T. & Nylander, A. (1996) 'Genetic and phenotypic parameters for age at starting to race and racing performance during early career in trotters', *Livestock Production Science*, 45, pp.63-68.

Safari, E., Fogarty, N.M. & Gilmour, A.R. (2005) 'A review of genetic parameter estimates for wool, growth, meat and reproduction traits in sheep', *Livestock Production Science*, 92, pp.271-289.

Salimetrics. (2013) *Saliva Collection and Handling Advice*. 3rd ed. Salimetrics, LLC.

Sánchez-Molano, E., Woolliams, J.A., Blott, S.C. & Wiener, P. (2014) 'Assessing the impact of genomic selection against hip dysplasia in the Labrador Retriever dog', *Journal of Animal Breeding and Genetics*, 131, pp.134-145.

Sanders, N. (1962) 'Lameness and allied conditions in the greyhound', *Australian Veterinary Journal*, 38, pp.239-243.

SAS Institute Inc. (2002-2010) *SAS Software* (version 9.3) [Computer program]. North Carolina: SAS Institute Inc.

Schaaf, O.R., Eaton-Wells, R. & Mitchell, R.A.S. (2009) 'Biceps Brachii and Brachialis Tendon of Insertion Injuries in Eleven Racing Greyhounds', *Veterinary Surgery*, 38, pp.825-833.

Schmidt, A., Biau, S., Möstl, E., Becker-Birck, M., Morillon, B., Aurich, J., Faure, J.M. & Aurich, C. (2010a) 'Changes in cortisol release and heart rate variability in sport horses during long-distance road transport', *Domestic Animal Endocrinology*, 38, pp.179-189.

Schmidt, A., Möstl, E., Wehnert, C., Aurich, J., Müller, J. & Aurich, C. (2010b) 'Cortisol release and heart rate variability in horses during road transport', *Hormones and Behavior*, 57, pp.209-215.

Schmidt, A., Aurich, J., Möstl, E., Müller, J. & Aurich, C. (2010c) 'Changes in cortisol release and heart rate and heart rate variability during the initial training of 3-year-old sport horses', *Hormones and Behavior*, 58, pp.628-636.

Schmidt, A., Hödl, S., Möstl, E., Aurich, J., Müller, J. & Aurich, C. (2010d) 'Cortisol release, heart rate, and heart rate variability in transport-naive horses during repeated road transport', *Domestic Animal Endocrinology*, 39, pp.205-213.

Schneider, H.P., Truex, R.C. & Knowles, J.O. (1964) 'Comparative observations of the hearts of mongrel and Greyhound dogs', *The Anatomical Record*, 149, pp.173-179.

Schneider, L.A., Delfabbro, P.H. & Burns, N.R. (2012) 'The influence of cerebral lateralisation on the behaviour of the racing greyhound', *Applied Animal Behaviour Science*, 141, pp.57-64.

Schoning, P.R. (1995) 'Endocardiosis and other heart-disease in greyhounds', *Journal of Veterinary Medicine Series A*, 42 (2), pp.99-104.

Schurink, A., Theunissen, M.C.J., Ducro, B.J., Bijma, P. & Grevenhof, E.M. van (2009) 'Identification of environmental factors affecting the speed of purebred Arabian racehorses in The Netherlands', *Livestock Science*, 125, pp.97-100.

Schwartz, E.B., Granger, D.A., Susman, E.J., Gunnar, M.R. & Laird, B. (1998) 'Assessing Salivary Cortisol in Studies of Child Development', *Child Development*, 69 (6), December, pp.1503-1513.

Scott, K.C., Hill, R.C., Lewis, D.D., Boning, A.J. & Sundstrom, D.A. (2001) 'Effect of α -tocopheryl acetate supplementation on vitamin E concentrations in Greyhounds before and after a race', *American Journal of Veterinary Research*, 62 (7), July, pp.1118-1120.

Seppälä, E.H., Koskinen, L.L.E., Gulløv, C.H., Jokinen, P., Karlskov-Mortensen, P., Bergamasco, L., Körberg, I., Baranowska, Cizinauskas, S., Oberbauer, A.M., Berendt, M., Fredholm, M. & Lohi, H. (2012) 'Identification of a Novel Idiopathic Epilepsy Locus in Belgian Shepherd Dogs', *PLOS One*, 7 (3), March, pp.1-11.

Serpell, J.A. & Hsu, Y.A. (2005) 'Effects of breed, sex and neuter status on trainability in dogs', *Anthrozoos*, 18 (3), pp.196-207.

Sewalem, A., Miglior, F. & Kistemaker, G.J. (2011) 'Short communication: Genetic parameters of milking temperament and milking speed in Canadian Holsteins', *Journal of Dairy Science*, 94 (1), pp.512-516.

Sharp, N.C.C. (2012) 'Animal athletes: a performance review', *Veterinary Record*, 171, pp.87-94.

Shearin, A.L. & Ostrander, E.A. (2010) 'Canine Morphology: Hunting for Genes and Tracking Mutations', *PLOS Biology*, 8 (3), March, pp.1-6.

Short, A.D., Kennedy, L.J., Forman, O., Barnes, A., Fretwell, N., Wiggall, R., Thomson, W. & Ollier, W.E.R. (2005) 'Canine DNA Subjected to Whole Genome Amplification is Suitable for a Wide Range of Molecular Applications', *Journal of Heredity*, 96 (7), pp.829-835.

Sicard, G.K., Short, K. & Manley, P.A. (1999) 'A survey of 4 injuries at five greyhound racing tracks', *Journal of Small Animal Practice*, 40, September, pp.428-432.

Signet (2015) *The national genetic evaluation service for cattle and sheep*. [Online] Available from: <http://www.signetfbc.co.uk/> (Accessed: 11 January, 2016).

Silvestre, A.M., Ginjaa, M.M.D., Ferreira, A.J.A. & Colaço, J. (2007) 'Comparison of estimates of hip dysplasia genetic parameters in Estrela Mountain Dog using linear and threshold models', *Journal of Animal Science*, 85, pp.1880-1884.

Simm, G. (1998) *Genetic Improvement of Cattle and Sheep*. Oxfordshire: CABI Publishing.

Singer, A., Ben-Yehuda, O., Ben-Ezea, Z. & Zaltzman, S. (1990) 'Multiple identical stress fractures in monozygotic twins. Case report', *Journal of Bone & Joint Surgery*, 72 (3), pp.444-445.

Smith, T.E. & French, J.A. (1997) 'Psychosocial Stress and Urinary Cortisol Excretion in Marmoset Monkeys (*Callithrix kuhli*)', *Physiology & Behaviour*, 62 (2), pp.225-232.

Smith, G.K., Mayhew, P.D., Kapatkin, A.S., McKelvie, P.J., Shofer, F.S. & Gregor, T.P. (2001) 'Evaluation of risk factors for degenerative joint disease associated with hip dysplasia in German Shepherd Dogs, Golden Retrievers, Labrador Retrievers, and Rottweilers', *Journal of the American Veterinary Medical Association*, 219 (12), December, pp.1719-1724.

Snow, D.H., Harris, R.C. & Stuttard, E. (1988) 'Changes in haematology and plasma biochemistry during maximal exercise in greyhounds', *Veterinary Record*, 123 (19), November, pp.48-489.

Sobczynska, M. & Lukaszewicz, M. (2004) 'Genetic parameters of racing merit of thoroughbred horses in Poland', *Journal of Animal Breeding and Genetics*, 121, pp.302-306.

Spencer, C.C.A., Su, Z., Donnelly, P. & Marchini, J. (2009) 'Designing Genome-Wide Association Studies: Sample Size, Power, Imputation, and the Choice of Genotyping Chip', *PLOS Genetics*, 5 (5), May, pp.1-13.

Stafford, K.J. (2007) 'Chapter 8: The Welfare of the Athletes; Greyhounds and Sled Dogs'. In: Stafford, K.J. *The Welfare of Dogs*. London: Springer.

Starkey, M.P., Scase, T.J., Mellersh, C.S. & Murphy, S. (2005) 'Dogs really are man's best friend — Canine genomics has applications in veterinary and human medicine!', *Briefings in Functional Genomics and Proteomics*, 4 (2), July, pp.112-128.

Stawski, R.S., Cichy, K.E., Piazza, J.R. & Almeida, D.M. (2013) 'Associations among daily stressors and salivary cortisol: Findings from the National Study of Daily Experiences', *Psychoneuroendocrinology*, 28, pp.2654-2665.

Stewart, I.D., Woolliams, J.A. & Brotherstone, S. (2010) 'Genetic evaluation of horses for performance in dressage competitions in Great Britain', *Livestock Science*, 128, pp.36-45.

Stewart, I.D., Woolliams, J.A. & Brotherstone, S. (2011) 'Genetic evaluations of traits recorded in British young horse tests', *Archiv Tierzucht*, 54 (5), pp.439-455.

Stock, K.F. & Distl, O. (2005) 'Evaluation of expected response to selection for orthopedic health and performance traits in Hanoverian Warmblood horses', *American Journal of Veterinary Research*, 66 (8), August, pp.1371-1379.

Stock, K.F. & Distl, O. (2010) 'Simulation study on the effects of excluding offspring information for genetic evaluation versus using genomic markers for selection in dog breeding', *Journal of Animal Breeding and Genetics*, 127, pp.42-52.

Stock, K.F., Klein, S., Tellhelm, B. & Distl, O. (2011) 'Genetic analyses of elbow and hip dysplasia in the German shepherd dog', *Journal of Animal Breeding and Genetics*, 128, pp.219-229.

Stracke, J., Bert, B., Fink, H. & Bohner, J. (2011) 'Assessment of stress in laboratory beagle dogs constrained by a Pavlov sling', *ALTEX*, 28 (4), pp.317-325.

Strohbach, C.A., Scofield, D.E., Nindl, B.C., Centi, A.J., Yanovich, R., Evans, R.K. & Moran, D.S. (2012) 'Female recruits sustaining stress fractures during military basic training demonstrate differential concentrations of circulating IGF-I system components: A preliminary study', *Growth Hormone & IGF Research*, 22, pp.151-157.

Stott, A.W., Coffey, M.P. & Brotherstone, S. (2005) 'Including lameness and mastitis in a profit index for dairy cattle', *Animal Science*, 80 (1), pp.41-52.

Strickler, T., Malone, T. & Garrett, W.E. (1990) 'The effects of passive warming on muscle injury', *The American Journal of Sports Medicine*, 18 (2), pp.141-145.

Sutter, N.B., Eberle, M.A., Parker, H.G., Pullar, B.J., Kirkness, E.F., Kruglyak, L. & Ostrander, E.A. (2004) 'Extensive and breed-specific linkage disequilibrium in *Canis familiaris*', *Genome Research*, 14, pp.2388-2396.

Svobodova, S., Blouin, C. & Langlois, B. (2005) 'Estimation of genetic parameters of Thoroughbred racing performance in the Czech Republic', *Animal Research*, 54, pp.499-509.

Svobodová, I., Chaloupková, H., Končel, R., Bartoš, L., Hradecká, L. & Jebavý, L. (2014) 'Cortisol and Secretory Immunoglobulin A Response to Stress in German Shepherd Dogs', *PLOS One*, 9 (3), March, pp.1-5.

Swan, A.A., Purvis, I.W. & Piper, L.R. (2008) 'Genetic parameters for yearling wool production, wool quality and body weight traits in fine wool Merino sheep', *Australian Journal of Experimental Agriculture*, 48, pp.1168-1176.

Sweeney, P.A. (1972) 'Some observations on behaviour of Greyhounds', *Journal of Small Animal Practice*, 13, pp.679-685.

Syme, D.A. (2005) 'Functional Properties of Skeletal Muscle', *Fish Physiology*, 23, pp.179-240.

Takeuchi, Y., Hashizume, C., Arata, S., Inoue-Murayama, M., Maki, T., Hart, B.L. & Mori, Y. (2008) 'An approach to canine behavioural genetics employing guide dogs for the blind', *Animal Genetics*, 40, pp.217-224.

Tan, H. & Wilson, A.M. (2011) 'Grip and limb force limits to turning performance in competition horses', *Proceedings of the Royal Society B*, 278, pp.2105-2111.

Täubert, H., Avena, D. & Simianer, H. (2007) 'Genetic analysis of racing performance in Irish greyhounds', *Journal of Animal Breeding and Genetics*, 124, pp.117-123.

Täubert, H. & Avena, D. (n.d.) *Quantitative Traits* [Online] Available from: http://www.greyhound-data.com/dir/536/Quantitative_Genetics.pdf (Accessed: 13 October 2013).

Taveira, R.Z. Mota, M.D.S. & Oliveira, H.N. (2004) 'Population parameters in Brazilian Thoroughbred', *Journal of Animal Breeding and Genetics*, 121, pp.384-391.

Taylor, D. (1997) 'Bone Maintenance and Remodeling: A Control System based on Fatigue Damage', *Journal of Orthopaedic Research*, 15 (4), July, pp.601-606.

Taylor, D. (1998) 'Fatigue of Bone and Bones: An Analysis Based on Stressed Volume', *Journal of Orthopaedic Research*, 16 (2), May, pp.163-169.

Teare, M.D. (2011) *Candidate Gene Association Studies*. In: Genetic Epidemiology. Ed: M.D. Teare. London: Humana Press.

Tharwat, M., Al-Sobayil, F. & Buczinski, S. (2013) 'Influence of racing on the serum concentrations of the cardiac biomarkers troponin I and creatine kinase myocardial band (CK-MB) in racing greyhounds', *The Veterinary Journal*, 197, pp.900-902.

Thiruvankadan, A.K., Kandasamy, N. & Panneerselvam, S. (2009a) 'Inheritance of racing performance of Thoroughbred horses', *Livestock Science*, 121, pp.308-326.

Thiruvankadan, A.K., Kandasamy, N. & Panneerselvam, S. (2009b) 'Inheritance of racing performance of trotter horses: An overview', *Livestock Science*, 124, pp.163-181.

Thompson, D.J., Cave, N.J., Bridges, J.P., Reuvers, K., Owen, M.C. & Firth, E.C. (2012) 'Bone volume and regional density of the central tarsal bone detected using computed tomography in a cross-sectional study of adult racing greyhounds', *New Zealand Veterinary Journal*, 60 (5), pp.278-284.

Thomson, P.C., Wilson, B.J., Wade, C.M., Shariflou, M.R., James, J.W., Tammen, I., Raadsma, H.W. & Nicholas, F.W. (2010) 'The utility of estimated breeding values for inherited disorders of dogs', *The Veterinary Journal*, 183, pp.243-244.

Toll, P.W., Pieschl, R.L. & Hand, M.S. (1992) 'The effect of dietary fat and carbohydrate on sprint performance in racing Greyhound dogs', In: *Proceedings. North American Veterinary Conference*, pp.1-3.

Toll, P.W., Gaehtgens, P., Neuhaus, D., Pieschl, R.L. & Fedde, M.R. (1995) 'Fluid, electrolyte, and packed cell volume shifts in racing greyhounds', *American Journal of Veterinary Research*, 56 (2), pp.227-232.

Tomlin, J.L., Lawes, T.J., Blunn, G.W., Goodship, A.E. & Muir, P. (2000) 'Fractographic Examination of Racing Greyhound Central (Navicular) Tarsal Bone Failure Surfaces Using Scanning Electron Microscopy', *Calcified Tissue International*, 67, pp.260-266.

Tontonoz, P. & Spiegelman, B.M. (2008) 'Fat and Beyond: The Diverse Biology of PPAR γ ', *Annual Review of Biochemistry*, 77, pp.289-312.

Tozaki, T., Miyake, T., Kakoi, H., Gawahara, H., Sugita, S., Hasegawa, T., Ishida, N., Hirota, K. & Nakano, Y. (2010) A genome-wide association study for racing performances in Thoroughbreds clarifies a candidate region near the MSTN gene', *Animal Genetics*, 41 (Suppl. 2), pp.28-35.

Tozaki, T., Hirota, K., Sugita, S., Ishida, N., Miyake, T., Oki, H. & Hasegawa, T. (2010) 'A genome-wide scan for tying-up syndrome in Japanese Thoroughbreds', *Animal Genetics*, 41 (Suppl. 2), pp.80-86.

Tozaki, T., Hill, E.W., Kakoi, H., Gawahara, H., Miyake, T., Sugita, S., Hasegawa, T., Ishida, N., Nakano, Y. & Kurosawa, M. (2011) 'A cohort study of racing performance in Japanese Thoroughbred racehorses using genome information on ECA18', *Animal Genetics*, 43, pp.42-52.

Tsai, K.L., Noorai, R.E., Starr-Moss, A.N., Quignon, P., Rinz, C.J., Ostrander, E.A., Steiner, J.M., Murphy, K.E. & Clark, L.A. (2012) 'Genome-wide association studies for multiple diseases of the German Shepherd Dog', *Mammalian Genome*, 23, pp.203-211.

Tuan, K., Wu, S. & Sennett, B. (2004) 'Stress Fractures in Athletes: Risk Factors, Diagnosis, and Management', *Orthopaedics*, 27 (6), June, pp.583-591.

Turner, J. (2010) *Animal Breeding, Welfare and Society*. London: Earthscan Ltd.

Uemura, E.E. (2015) *Autonomic Nervous System*. In: Dukes' Physiology of Domestic Animals. 13th ed. Eds: W.O. Reece, H.H. Erickson, J.P. Goff & E.E. Uemura. Ames, Iowa: Wiley Blackwell.

Usherwood, J.R. & Wilson, A.M. (2005) 'No force limit on greyhound sprint speed', *Nature*, 438 (8), pp.753-754.

Vainionpää, M., Tienhaara, E.P., Raekallio, M., Junnila, J., Snellman, M. & Vainio, O. (2012) 'Thermographic Imaging of the Superficial Temperature in Racing Greyhounds before and after the Race', *The Scientific World Journal*, 2012.

Välimäki, V.V., Alfthan, H., Lehmuskallio, E., Löyttyniemi, E., Sahi, T., Suominen, H. & Välimäki, M.J. (2005a) 'Risk factors for clinical stress fractures in male military recruits: A prospective cohort study', *Bone*, 37, pp.267-273.

Välimäki, V.V., Piippo, K., Välimäki, S., Löyttyniemi, E., Kontula, K. & Välimäki, M.J. (2005b) 'The relation of the XbaI and PvuII polymorphisms of the estrogen receptor gene and the CAG repeat polymorphism of the androgen receptor gene to peak bone mass and bone turnover rate among young healthy men', *Osteoporosis International*, 16, pp.1633-1640.

Vaughan, L.C. (1969) 'Gracilis Muscle Injury in Greyhounds', *Journal of Small Animal Practice*, 10, pp.363-375.

Veisser, I. & Boissy, A. (2007) 'Stress and welfare: two complimentary concepts that are intrinsically related to the animal's point of view', *Physiology & Behaviour*, 92, pp.429-433.

Verhoeven, G., Fortrie, R., Ryssen, B. van & Coopman, F. (2012) 'Worldwide Screening for Canine Hip Dysplasia: Where Are We Now?', *Veterinary Surgery*, 41, pp.10-19.

Viklund, Å, Hellsten, E. Thorén, Näsholm, A., Strandberg, E. & Philipsson. (2008) 'Genetic parameters for traits evaluated at field tests of 3- and 4-year-old Swedish Warmblood horses', *Animal*, 2 (12), pp.1832-1841.

Viklund, Å, Braam, Å., Näsholm, A., Strandberg, E. & Philipsson, J. (2010) 'Genetic variation in competition traits at different ages and time periods and correlations with traits at field tests of 4-year-old Swedish Warmblood horses', *Animal*, 4 (5), pp.682-691.

Vila, C. & Leonard, J.A. (2012) 'Chapter 1: Canid Phylogeny and Origin of the Domestic Dog'. In: Ostrander, E.A. & Ruvinsky, A. (eds.) *The Genetics of the Dog*. 2nd ed. Oxfordshire: CABI Publishing.

Villela, L.C.V., Mota, M.D.S. & Oliveira, H.N. (2002) 'Genetic parameters of racing performance traits of Quarter horses in Brazil', *Journal of Animal Breeding and Genetics*, 119, pp.229-234.

Vincent, I.C. & Michell, A.R. (1992) 'Comparison of cortisol concentrations in saliva and plasma of dogs', *Research in Veterinary Science*, 53, pp.342-345.

Visscher, P.M., Thompson, R. & Hill, W.G. (1991) 'Estimation of genetic and environmental variances for fat yield in individual herds and an investigation into heterogeneity of variance between herds', *Livestock Production Science*, 28, pp.273-290.

Visscher, P.M., Hill, W.G. & Wray, N.R. (2008) 'Heritability in the genomics era — concepts and misconceptions', *Nature Reviews Genetics*, 9, April, pp.255-266.

Von Borell, E., Dobson, H. & Prunier, A. (2007) 'Stress, behaviour and reproductive performance in female cattle and pigs', *Hormones and Behavior*, 52, pp.130-138.

Vostrý, L., Čapková, Z., Příbyl, J. & Mach, K. (2011) 'Analysis of Czech cold-blooded horses: genetic parameters, breeding value and the influence of inbreeding depression on linear description of conformation and type characters', *Czech Journal of Animal Science*, 56 (5), pp.217-230.

Vries, M.J. de, Waaij, E.H. van der & Arendok, J.A.M van (1998) 'Estimation of genetic parameters for litter size in sheep: a comparison of a repeatability and a multivariate model', *Animal Science*, 66 (3), pp.685-688.

Wakshlag, J., Sneddon, K. & Reynolds, A. (2004) 'Biochemical and Metabolic Changes due to Exercise in Sprint-Racing Sled Dogs: Implications for Postexercise Carbohydrate Supplements and Hydration Management', *Veterinary Therapeutics*, 5 (1).

Wakshlag, J.J., Stokol, T., Geske, S.M., Greger, C.E., Angle, C.T. & Gillette, R.L. (2010) 'Evaluation of exercise-induced changes in concentrations of C-reactive protein and serum biochemical values in sled dogs completing a long-distance endurance race', *American Journal of Veterinary Research*, 71 (10), October, pp.1207-1213.

Wall, E., Brotherstone, S., Woolliams, J.A., Banos, G. & Coffey, M.P. (2003) 'Genetic Evaluation of Fertility Using Direct and Correlated Traits', *Journal of Dairy Science*, 86 (12), pp.4093-4102.

Wallin, L., Strandberg, E. & Philipsson, J. (2003) 'Genetic correlations between field test results of Swedish Warmblood Riding Horses as 4-year-olds and lifetime performance results in dressage and show jumping', *Livestock Production Science*, 82, pp.61-71.

Wan, Y. (2010) 'PPAR γ in bone homeostasis', *Trends in Endocrinology and Metabolism*, 21 (12), pp.722-728.

Wangdee, C., Leegwater, P.A.J., Heuven, H.C.M., Steenbeek, F.G. van, Meutstege, F.J., Meij, B.P. & Hazewinkel, H.A.W. (2014) 'Prevalence and genetics of patellar luxation in Kooiker dogs', *The Veterinary Journal*. In Press. Available from: <https://www.sciencedirect.com> (Accessed: 30 July 2014).

Warden, S.J., Creaby, M.W., Bryant, A.L. & Crossley, K.M. (2007) 'Stress fracture risk factors in female football players and their clinical implications', *British Journal of Sports Medicine*, 41 (Suppl 1), pp.i38-i43.

Wayne, R.K. & Ostrander, E.A. (2007) 'Lessons learned from the dog genome', *Trends in Genetics*, 23 (11), pp.557-567.

Weber, A., Stamer, E., Junge, W. & Thaller, G. (2013) 'Genetic parameters for lameness and claw and leg diseases in dairy cows', *Journal of Dairy Science*, 96 (5), pp.3310-3318.

Webster, J. (1994). *Animal welfare: A cool eye towards Eden*. Oxford: Blackwell Publishing.

Webster, J. (2005). *Animal welfare: Limping towards Eden*. Oxford: UFAW/Wiley-Blackwell.

Wellcome Trust Case Control Consortium (2007) 'Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls', *Nature*, 447 (7145), pp.661-678.

The Welfare of Racing Greyhounds Regulations 2010 (England). SI 2010/543.
London: The Stationary Office Ltd.

Wendelburg, K., Dee, J., Kaderly, R., Dee, L. & Eaton-Wells, R. (1988) 'Stress Fractures of the Acetabulum in 26 Racing Greyhounds', *Veterinary Surgery*, 17 (3), pp.128-134.

Wentz, L., Liu, P.Y., Haymes, E. & Ilich, J.Z. (2011) 'Females Have a Greater Incidence of Stress Fractures Than Males in Both Military and Athletic Populations: A Systemic Review', *Military Medicine*, 176 (4), April, pp.420-430.

Weyand, P.G., Sandell, R.F., Prime, D.N.L & Bundle, M.W. (2010) 'The biological limits to running speed are imposed from the ground up', *Journal of Applied Physiology*, 108, pp.950-961.

The Whippet Club. (2016) *The Whippet Club*. [Online] Available from: <http://www.thewhippetclub.com/> (Accessed 1 January 2016).

Wilbe, M., Jokinen, P., Truvé, K., Seppala, E.H., Karlsson, E.K., Biagi, T., Hughes, A., Bannasch, D., Andersson, G., Hansson-Hamlin, H., Lohi, H. & Lindblad-Toh, K. (2010) 'Genome-wide association mapping identifies multiple loci for a canine SLE-related disease complex', *Nature Genetics*, 42 (3), March, pp.250-255.

Willham, R.L. & Wilson, D.E. (1991) 'Genetic predictions of racing performance in quarter horses', *Journal of Animal Science*, 69, pp.3891-3894.

Williams, S.B., Wilson, A.M., Rhodes, L., Andrews, J. & Payne, R.C. (2008a) 'Functional anatomy and muscle moment arms of the pelvic limb of an elite sprinting athlete: the racing greyhound (*Canis familiaris*)', *Journal of Anatomy*, 213, pp.361-372.

Williams, S.B., Wilson, A.M., Daynes, J., Peckham, K. & Payne, R.C. (2008b) 'Functional anatomy and muscle moment arms of the thoracic limb of an elite sprinting athlete: the racing greyhound (*Canis familiaris*)', *Journal of Anatomy*, 213, pp.373-382.

Williams, S.B., Usherwood, J.R., Jespers, K., Channon, A.J. & Wilson, A.M. (2009a) 'Exploring the mechanical basis for acceleration: pelvic limb locomotor function during accelerations in racing greyhounds (*Canis familiaris*)', *The Journal of Experimental Biology*, 212, pp.550-565.

Williams, S.B., Tan, H., Usherwood, J.R. & Wilson, A.M. (2009b) 'Pitch then power: limitations to acceleration in quadrupeds', *Biology Letters*, 5, pp.610-613.

Williamson, S.A. & Beilharz, R.G. (1996) 'Heritabilities of racing performance in Thoroughbreds: a study of Australian data', *Journal of Animal Breeding and Genetics*, 113, pp.505-524.

Williamson, S.A. & Beilharz, R.G. (1998) 'The inheritance of speed, stamina and other racing performance characters in the Australian Thoroughbred', *Journal of Animal Breeding and Genetics*, 115, pp.1-16.

Willis, M.B. (1997) 'A review of the progress in canine hip dysplasia control in Britain', *Journal of the American Veterinary Medical Association*, 210, pp.1480-1482.

Wilson, B.J., Nicholas, F.W., James, J.W., Wade, C.M., Tammen, I., Raadsma, H.W., Castle, K. & Thomson, P.C. (2011a) 'Symmetry of hip dysplasia traits in the German Shepherd Dog in Australia', *Journal of Animal Breeding and Genetics*, 128, pp.230-243.

Wilson, B., Nicholas, F.W. & Thomson, P.C. (2011b) 'Selection against canine hip dysplasia: Success or failure?', *The Veterinary Journal*, 189, pp.160-168.

Wilson, B.J., Nicholas, F.W., James, J.W., Wade, C.M., Tammen, I., Raadsma, H.W., Castle, K. & Thomson, P.C. (2012) 'Heritability and Phenotypic Variation of Canine Hip Dysplasia Radiographic Traits in a Cohort of Australian German Shepherd Dogs', *PLOS One*, 7 (6), pp.1-12.

Wingfield, C., Amis, A.A., Stead, A.C. & Law, H.T. (2000a) 'Comparison of the biomechanical properties of rottweiler and racing greyhound cranial cruciate ligaments', *Journal of Small Animal Practice*, 41, July, pp.303-307.

Wingfield, C., Amis, A.A., Stead, A.C. & Law, H.T. (2000b) 'Cranial cruciate stability in the rottweiler and racing greyhound: an in vitro study', *Journal of Small Animal Practice*, 41, May, pp.193-197.

Wirth, K.E. & Rein, D.B. (2008) 'The Economic Costs and Benefits of Dog Guides for the Blind', *Ophthalmic Epidemiology*, 15, pp.92-98.

Wittert, G.A., Livesey, J.H., Espiner, E.A. & Donald, R.A. (1996) 'Adaptation of the hypothalamopituitary adrenal axis to chronic exercise stress in humans', *Medicine & Science in Sports & Exercise*, 28 (8), August, pp.1015-1019.

Wood, S.H., Ke, X. & Nuttall, T. (2009) 'Genome-wide association analysis of canine atopic dermatitis and identification of disease related SNPs', *Immunogenetics*, 61, pp.761-772.

Woolliams, J.A., Lewis, T.W. & Blott, S.C. (2011) 'Canine hip and elbow dysplasia in UK Labrador retrievers', *The Veterinary Journal*, 189, pp.169-176.

World Organisation for Animal Health. (2016) *World Organisation for Animal Health (OIE)*. [Online] Available from: <http://www.oie.int/> (Accessed 1 November 2016)

Yanovich, R., Milgrom, R., Friedman, E. & Moran, D.S. (2011) 'Androgen Receptor CAG Repeat Size is Associated with Stress Fracture Risk', *Clinical Orthopaedics and Related Research*, 469 (10), October, pp.2925-2931.

Yanovich, R., Friedman, E., Milgrom, R., Oberman, B., Freedman, L. & Moran, D.S. (2012) 'Candidate gene analysis in Israeli soldiers with stress fractures', *Journal of Sports Science and Medicine*, 11, pp.147-155.

Yeates, J.W. (2010) 'Death is a Welfare Issue', *Journal of Agricultural & Environmental Ethics*, 23, pp.229-241.

Yokoyama, J.S., Erdman, C.A. & Hamilton, S.P. (2010) 'Array-Based Whole-Genome Survey of Dog Saliva DNA Yields High Quality SNP Data', *PLOS One*, 5 (5), May, pp.1-7.

Zaldívar-Lopez, S., Marin, L.M., Iazbik, M.C., Westendorf-Stingle, N., Hensley, S. & Couto, C.G. (2011) 'Clinical pathology of Greyhounds and other sighthounds', *Veterinary Clinical Pathology*, 40 (4), pp.414-425.

Zebarth, B.J. & Sheard, R.W. (1985) 'Impact and shear resistance of turfgrass racing surfaces for thoroughbreds', *American Journal of Veterinary Research*, 46, pp.778-784.

Zhang, Z., Zhu, L., Sandler, J., Friedenber, S.S., Egelhoff, J., Williams, A.J., Dykes, N.L., Hornbuckle, W., Krotscheck, U., Moise, N.S., Lust, G. & Todhunter, R.J. (2009) 'Estimation of heritabilities, genetic correlations, that collectively define hip dysplasia in dogs and breeding values of four traits', *American Journal of Veterinary Research*, 70 (4), pp.483-492.

Zhao, X., Onteru, S., Saatchi, M., Garrick, D. & Rothschild, M. (2013) 'A genome-wide association study for canine cryptorchidism in Siberian Huskies', *Journal of Animal Breeding and Genetics*, 131, pp.202-209.

Zhong, z., Zylstra-Diegel, C., Schumacher, C.A., Baker, J.J., Carpenter, A.C., Rao, S., Yao, W., Guan, M., Helms, J.A., Lane, N.E., Lang, R.A. & Williams, B.O. (2012) 'Wntless functions in mature osteoblasts to regulate bone mass', *Proceedings of the National Academy of Sciences of the United States of America*, 109 (33), August, pp.E2197-E2204.

Zhu, L., Zhang, Z., Friedenber, S., Jung, S.-W., Phavaphutanon, J., Vernier-Singer, M., Corey, E., Mateescu, R., Dykes, N., Sandler, J., Acland, G., Lust, G. & Todhunter, R. (2009) 'The long (and winding) road to gene discovery for canine hip dysplasia', *The Veterinary Journal*, 181, pp.97-110.

Ziegler, T.E., Scheffler, G. & Snowdon, C.T. (1995) 'The Relationship of Cortisol Levels to Social Environment and Reproductive Functioning in Female Cotton-Top Tamarins, *Saguinus oedipus*', *Hormones and Behavior*, 29, pp.407-424.

Zink, V., Štipková, M. & Lassen, J. (2011) 'Genetic parameters for female fertility, locomotion, body condition score, and linear type traits in Czech Holstein cattle', *Journal of Dairy Science*, 94 (10), pp.5176-5182.

Zondervan, K.T. (2011) 'Chapter 3: Genetic Association Study Design'. In: Zeggini, E. & Morris, A. (eds.) *Analysis of Complex Disease Association Studies' Book*. London: Elsevier Inc.

Appendices

Appendix 1: British Racing Greyhound Pedigree and Performance Database, Genetic Analysis and Estimated Breeding Values.

See disc.

Appendix 2a: Details of the module 'Check_Dog_Table' (Microsoft Access Visual Basic utility program) written to facilitate data checking and maintenance of the Greyhound Pedigree and Performance Database tables.

Module 'Check_Dog_Table' contained one subroutine that checked the Performance Data table against the Dog Data table, to determine whether corresponding Greyhound ID numbers were present and to list any discrepancies:

```
Sub Check_Dog_Table()
```

```
'This subroutine checks the Performance Data against the Dog Data  
table, determining if there  
'is a corresponding Dog ID.
```

```
'Currently all dogs accounted for. Number of unique dogs in  
Performance Data table = 52826
```

```
Dim db As DAO.Database  
Dim rs_PD As DAO.Recordset  
Dim ID, OldID, DogCount, Count As Long  
Dim n As Integer  
Dim sql_query As String
```

```
Set db = CurrentDb  
sql_query = "SELECT [Performance Data].[GH Data ID] FROM  
[Performance Data] ORDER BY [Performance Data].[GH Data ID];"
```

```

Set rs_PD = db.OpenRecordset(sql_query)
rs_PD.MoveFirst
OldID = 0
Count = 0
DogCount = 0
Do While Not rs_PD.EOF Or rs_PD.BOF
    ID = rs_PD("GH Data ID")
    If ID <> OldID Then
        n = DCount("[GH Data ID]", "Dog Data", "[GH Data ID] = " & ID)
        If n = 0 Then
            Debug.Print ID
            'Debug.Print Trim$(ID)
            Count = Count + 1
        End If
        OldID = ID
        DogCount = DogCount + 1
    End If
    'DoEvents
    rs_PD.MoveNext
Loop

Debug.Print Count, DogCount

Set rs_PD = Nothing

```

```
End Sub
```

Appendix 2b: Details of the module 'Check_Pedigree_Table'

(Microsoft Access Visual Basic utility program) written to facilitate data checking and maintenance of the Greyhound Pedigree and Performance Database tables.

Module 'Check_Pedigree_Table' contained one subroutine that checked the Phenotypic Data table against the Pedigree Data table, to determine whether corresponding Greyhound ID numbers were present and to list any discrepancies:

```
Sub Check_Pedigree_Table()  
'This subroutine checks the Dog Data against the Pedigree Data table,  
determining if there  
'is a corresponding Dog ID.  
  
Dim db As DAO.Database  
Dim rs_DD, rs_PD As DAO.Recordset  
Dim ID, Count As Long  
Dim n As Integer  
Dim sql_query As String  
Dim Mod_Pedigree_Table As Boolean  
  
Mod_Pedigree_Table = False  
  
Set db = CurrentDb  
If Mod_Pedigree_Table = True Then Set rs_PD =  
db.OpenRecordset("Pedigree Data")  
  
sql_query = "SELECT [Dog Data].[Greyhound ID] FROM [Dog Data]  
ORDER BY [Dog Data].[Greyhound ID];"  
  
Set rs_DD = db.OpenRecordset(sql_query)  
rs_DD.MoveFirst  
  
Count = 0  
DogCount = 0  
  
Do While Not rs_DD.EOF Or rs_DD.BOF  
ID = rs_DD("Greyhound ID")  
  
n = DCount("[Greyhound ID]", "Pedigree Data", "[Greyhound ID] = " &  
ID)
```

```

    If n = 0 Then
        Debug.Print ID
        If Mod_Pedigree_Table = True Then
            rs_PD.AddNew
            rs_PD.Fields("Greyhound ID") = ID
            rs_PD.Fields("Sire ID") = 0
            rs_PD.Fields("Dam ID") = 0
            rs_PD.Update
        End If
        'Debug.Print Trim$(ID)
        Count = Count + 1
    End If

    'DoEvents
    rs_DD.MoveNext
Loop

Debug.Print Count

Set rs_DD = Nothing
Set rs_PD = Nothing

End Sub

```

Appendix 2c: Details of the module 'Check_Performance_ID'
(Microsoft Access Visual Basic utility program) written to facilitate data checking
and maintenance of the Greyhound Pedigree and Performance Database tables.

Module 'Check_Performance_ID' checked the Performance Data table against the Race Data table, to ensure they both contained corresponding values for RaceID:

```
Sub Check_Performance_ID()  
  
    'This subroutine checks the Race Data against the Performance Data  
    'table, determining if there  
    'is a corresponding Race ID.  
  
    Dim db As DAO.Database  
    Dim rs_RD As DAO.Recordset  
    Dim RaceID, Count As Long  
    Dim n, nDogs As Integer  
  
    Set db = CurrentDb  
    Set rs_RD = db.OpenRecordset("Race Data")  
    rs_RD.MoveFirst  
  
    Count = 0  
    Do While Not rs_RD.EOF Or rs_RD.BOF  
        ID = rs_RD("RaceID")  
        nDogs = rs_RD("NoDogs")  
  
        n = DCount("[RaceID]", "Performance Data", "[RaceID] = " & ID)  
        If n <> nDogs Then  
            Debug.Print ID; nDogs; n  
            'Debug.Print Trim$(ID)  
            Count = Count + 1  
        End If  
  
        'DoEvents  
        rs_RD.MoveNext  
    Loop  
  
    Debug.Print Count  
  
    Set rs_RD = Nothing  
End Sub
```

Appendix 3: The Structured Query Language - 'Check_Pedigree_Data' query performed in Microsoft Access, in order to cross-check records in the Pedigree Data table and Phenotypic Data table of the Greyhound Pedigree and Performance Database. The query was used to determine whether any errors in dates of birth were present.

```
SELECT [Pedigree Data].[Greyhound ID], [Dog Data].Name, [Dog Data].DOB, [Dog  
Data_1].[Greyhound ID], [Dog Data_1].Link, [Dog Data_2].[Greyhound ID], [Dog  
Data_2].Link, [Dog Data_1].DOB, [Dog Data_2].DOB, IIf([Dog Data_1.DOB]>[Dog  
Data.DOB],"Error","") AS [Sire DOB Error], IIf([Dog Data_2.DOB]>[Dog  
Data.DOB],"Error","") AS [Dam DOB Error]  
FROM (([Dog Data] INNER JOIN [Pedigree Data] ON [Dog Data].[Greyhound ID] =  
[Pedigree Data].[Greyhound ID]) INNER JOIN [Dog Data] AS [Dog Data_1] ON  
[Pedigree Data].[Sire ID] = [Dog Data_1].[Greyhound ID]) INNER JOIN [Dog Data] AS  
[Dog Data_2] ON [Pedigree Data].[Dam ID] = [Dog Data_2].[Greyhound ID]  
ORDER BY IIf([Dog Data_1.DOB]>[Dog Data.DOB],"Error","") DESC , IIf([Dog  
Data_2.DOB]>[Dog Data.DOB],"Error","") DESC;
```


Appendix 4: Information sheet for Greyhound owners or trainers considering donation of a saliva sample from a dog.



Information for Greyhound Owners or Trainers Considering Donation of a Saliva Sample from a Dog

We would like to ask for your help. At the University of Liverpool we carry out research into the health, welfare and performance of Racing Greyhounds. We are carrying out a genetics study to investigate whether some Greyhounds are prone to certain types of racing injuries. We use state of the art genome sequencing techniques to identify differences in the genetic code between injured and uninjured dogs. This will help us to understand the risk factors for different injuries and, eventually, to find ways to reduce them. When we have completed the study we will communicate everything we learn back to the Greyhound community via trainers' forums, Greyhound Vets and the GBGB.

To carry out this research, we need to take saliva samples from dogs using special collection kits. The procedure is very simple and does not cause any harm or distress. We place two small sponges into the dog's mouth for 30-60 seconds and collect the saliva. These samples give us information about the genetics of the dog. We also store part of the samples for use in future projects that will benefit greyhound health and welfare. This way we can gain the maximum benefit from the donation.

We would be very grateful if you will agree to donate a saliva sample from your dog. Your involvement is completely voluntary. Your identity, and that of your dog, will remain anonymous and will not be disclosed. Only the people working on this project will have access to individual data.

If you are willing to help us, we need you to sign a simple form (attached) to confirm you have agreed to the donation and you understand what the samples will be used for. We are delighted to talk to you about our work and we really appreciate all of your comments and suggestions. Thank you very much for considering helping us.

Yours sincerely,

Ruth Dockerty

Ruth Dockerty BVSc MSc MRCVS

If you have any questions or concerns about this study, please contact Ruth Dockerty on: ***** or *****

Appendix 5: Information sheet for Greyhound owners or trainers considering donation of a tissue sample from a dog.



Information for Greyhound Owners or Trainers Considering Donation of a Tissue Sample from a Dog

We would like to ask for your help. At the University of Liverpool we carry out research into the health, welfare and performance of Racing Greyhounds. We are carrying out a genetics study to investigate whether some Greyhounds are prone to certain types of racing injuries. We use state of the art genome sequencing techniques to identify differences in the genetic code between injured and uninjured dogs. This will help us to understand the risk factors for different injuries and, eventually, to find ways to reduce them. When we have completed the study we will communicate everything we learn back to the Greyhound community via trainers' forums, Greyhound Vets and the GBGB.

To carry out this research, we need to take postmortem skin tissues samples from dogs that have been euthanased after injury. We also store part of the samples for use in future projects that will benefit greyhound health and welfare. This way we can gain the maximum benefit from the donation.

We would be very grateful if you will agree to donate a tissue sample from your dog. Your involvement is completely voluntary. Your identity, and that of your dog, will remain anonymous and will not be disclosed. Only the people working on this project will have access to individual data.

If you are willing to help us, we need you to sign a simple form (attached) to

confirm you have agreed to the donation and you understand what the samples will be used for. We are delighted to talk to you about our work and we really appreciate all of your comments and suggestions. Thank you very much for considering helping us.

Yours sincerely,



Ruth Dockerty BVSc MSc MRCVS

If you have any questions or concerns about this study, please contact Ruth Dockerty on: ***** or *****

Appendix 6: Consent form for the donation of a saliva sample from a Greyhound.



Consent for the Donation of a Saliva Sample from a Greyhound

1. I confirm that I have read and have understood the information sheet for the donation of a saliva sample from a Greyhound. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐
2. I understand that my participation is completely voluntary and if I decline to participate this will not affect the veterinary treatment of my animal. ☐
3. I understand that under the Data Protection Act, I can ask for access to the information I provide at any time and I can request the destruction of that information. I understand that I may refuse to answer particular questions and my individual privacy will be maintained in all published and written data from the study. ☐
4. I agree to donate the saliva sample of my dog for research that will benefit greyhound welfare. ☐

If you agree to these conditions, please sign below:

Owner or Trainer Name (please print): _____

Signature: _____

Date: _____

Contact: Ruth Dockerty BVSc MSc MRCVS
University of Liverpool – School of Veterinary Science
Tel: *****
Email: *****

Appendix 7: Consent form for the donation of a tissue sample from a Greyhound.



Consent for the Donation of a Tissue Sample from a Greyhound

1. I confirm that I have read and have understood the information sheet for the donation of a tissue sample from a Greyhound. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐
2. I understand that my participation is completely voluntary and if I decline to participate this will not affect the veterinary treatment of my animals. ☐
3. I understand that under the Data Protection Act, I can ask for access to the information I provide at any time and I can request the destruction of that information. I understand that I may refuse to answer particular questions and my individual privacy will be maintained in all published and written data from the study. ☐
4. I agree to donate the tissue sample from my dog for research that will benefit greyhound welfare. ☐

If you agree to these conditions, please sign below:

Owner or Trainer Name (please print): _____

Signature: _____

Date: _____

Contact: Ruth Dockerty BVSc MSc MRCVS
University of Liverpool – School of Veterinary Science
Tel: *****
Email: *****

Appendix 8: Form used to obtain Greyhound phenotypic data from the owner or trainer donating a saliva sample for use in the Genome-Wide Association Study.



Sample ID:	Date of Sample Collection:
Name of Trainer:	
Kennels Address:	
Phone No:	

ANIMAL DETAILS

Greyhound Racing Name:

Ear Tattoo:

Sex: M F **Neutered:** Y N

DOB: /...../..... **Weight (kg):**

Place of Birth: **Time at Kennel:** ym

Diet:

.....

.....

.....

Exercise & Training:

.....
.....
.....

History of Injuries:

.....
.....
.....
.....

Other Medical Conditions:

.....
.....

Appendix 9: Phenotypic Data for Greyhounds in the GWA Study.

Where: *Sample ID*, ID number of the dog; *Sex*, male (M) or female (F); *Date of Birth*, birth date of the dog; *Place of Birth*, country of birth; *Weight*, body weight of the dog in kilograms; *Stress Fracture*, location of the stress fracture sustained by the dog (cases); *No. Races to Fracture*, total number of races ran by the dog before fracture occurred (cases); *No. Races Ran*, total number of races ran by the dog without fracture occurring, to date of sample collection (controls); *Trainer*, ID number of the Greyhound trainer; *Date Sample Collection*, date saliva sample collected from the dog; *DNA*, the concentration of DNA extracted from the saliva sample, in nanograms per microlitre.

Appendix 9: Phenotypic Data for Greyhounds in the GWA Study (Cases):

Sample ID	Sex	Date of Birth	Place of Birth	Weight (kg)	Stress Fracture	No. Races to Fracture	Trainer	Date Sample Collection	DNA (ng/ul)
S001	M	01/08/2009	Ireland	33	Right Hock	49	1	22/06/2012	16
S006	F	13/09/2009	Ireland	29	Right Hock	39	1	22/06/2012	16.8
S025	F	18/05/2010	UK	26	Right MT	17	2	12/07/2012	43.4
S026	F	01/07/2009	Ireland	29	Left MT	35	2	12/07/2012	74.8
S029	F	01/08/2008	Ireland	28.4	Right Hock	43	3	13/07/2012	18.3
S044	F	23/04/2008	UK	Unknown	Hock (<i>Unknown limb</i>)	46	4	16/07/2012	63
S047	M	14/06/2009	Ireland	Unknown	Hock (<i>Unknown limb</i>)	27	4	16/07/2012	109
S053	F	01/11/2008	Unknown	Unknown	Hock (<i>Unknown limb</i>)	44	4	16/07/2012	19.5
S073	F	15/06/2008	Ireland	28.2	MT (<i>Unknown limb</i>)	43	5	17/07/2012	21.5
S079	M	12/03/2010	Ireland	33	Left Hock	10	6	23/07/2012	14.6
S081	M	01/10/2009	Ireland	36	Right & Left Hock	24	6	23/07/2012	18.5
S085	M	18/08/2009	Ireland	32.5	Right Hock	27	6	23/07/2012	49.4
S088	M	16/02/2009	Ireland	34	Left Hock	23	6	23/07/2012	14.4
S095	M	01/04/2010	Ireland	31	Left MT	9	6	23/07/2012	30.8
S097	M	02/03/2009	Ireland	34.5	Right Hock	49	6	23/07/2012	19
S106	M	01/11/2009	Ireland	32.5	Right Hock	18	8	31/07/2012	20.4
S107	F	23/04/2006	Ireland	29	Right Hock	34	8	31/07/2012	52.7
S108	M	01/06/2010	Ireland	32.3	Left MC	21	8	31/07/2012	19.7
S122	M	19/05/2009	Ireland	31	Right Hock	43	9	13/08/2012	16
S125	M	01/11/2008	Ireland	31	Right Hock	32	9	13/08/2012	28.9
S150	F	01/12/2007	Ireland	26.6	Right Hock	50	10	04/09/2012	19.6
S171	F	01/01/2010	UK	28	Right Hock	30	11	06/09/2012	31
S184	F	18/07/2009	Ireland	25.5	Hock (<i>Unknown limb</i>)	50	12	27/09/2012	21.8
S185	M	01/07/2008	Unknown	Unknown	Hock (<i>Unknown limb</i>)	40	5	27/09/2012	25.3

Appendix 9: Phenotypic Data for Greyhounds in the GWA Study (Controls):

Sample ID	Sex	Date of Birth	Place of Birth	Weight (kg)	No. Races Ran	Trainer	Date Sample Collection	DNA (ng/ul)
S033	M	10/01/2008	Ireland	33.7	105	3	13/07/2012	40.4
S036	F	08/05/2008	UK	<i>Unknown</i>	126	4	16/07/2012	115
S058	M	02/10/2003	Ireland	<i>Unknown</i>	99	4	16/07/2012	42.3
S066	M	10/02/2008	UK	<i>Unknown</i>	130	4	16/07/2012	55.8
S076	M	10/03/2008	Ireland	35	102	5	17/07/2012	52
S111	F	01/01/2008	UK	27	118	8	31/07/2012	60.3
S123	M	01/11/2007	Ireland	40	112	9	13/08/2012	77.8
S148	M	01/08/2004	<i>Unknown</i>	33	151	13	03/09/2012	118
S146	F	12/09/2007	Ireland	27	93	13	03/09/2012	65.1
S116	F	11/03/2001	UK	26	87	8	31/07/2012	73
S180	M	01/10/2008	<i>Unknown</i>	30	101	12	27/09/2012	20.9
S143	F	01/01/2009	Ireland	25	96	13	03/09/2012	22.8
S193	F	01/03/2005	<i>Unknown</i>	<i>Unknown</i>	128	15	27/09/2012	17.5
S178	M	01/07/2009	Ireland	30.5	125	12	27/09/2012	20.9
S060	F	01/08/2005	Ireland	<i>Unknown</i>	135	4	16/07/2012	32.7
S043	M	01/06/2008	Ireland	<i>Unknown</i>	108	4	16/07/2012	25
S177	F	01/09/2007	<i>Unknown</i>	24.5	118	12	27/09/2012	30.8
S105	F	01/10/2006	Ireland	<i>Unknown</i>	144	4	16/07/2012	33.7
S082	F	06/10/2009	Ireland	27	118	6	23/07/2012	43.5
S074	F	01/08/2008	UK	28	83	5	17/07/2012	31.5
S147	M	01/05/2009	<i>Unknown</i>	33	98	13	03/09/2012	41.1
S054	F	05/07/2006	Ireland	<i>Unknown</i>	160	4	16/07/2012	26.8
S086	M	01/11/2008	UK	35.9	98	6	23/07/2012	26
S109	M	01/09/2008	Ireland	31.1	102	8	31/07/2012	26.2

Appendix 10: Greyhound Phenotypic Data, Heart Rates and Cortisol Measurements.

Sample ID	Dog ID	Sample Type	Sex	Age (months)	Race No.	Time of Race	Race Dist (m)	No. Dogs	Trap No.	Adjusted Rank	Journey Distance (km)	Owner/Trainer	HR (bpm)	Cortisol (ng/ml)
1	1	A	F	18	1	7.30pm	265	4	2	4.33	45.22	1	152	7.178
2	1	B	F	18	1	7.30pm	265	4	2	4.33	45.22	1	200	28.498
3	2	A	M	72	1	7.30pm	265	4	3	2.67	40.39	2	124	4.277
4	2	B	M	72	1	7.30pm	265	4	3	2.67	40.39	2	212	10.280
5	3	A	F	18	1	7.30pm	265	4	6	6.00	45.22	1	150	15.730
6	3	B	F	18	1	7.30pm	265	4	6	6.00	45.22	1	180	21.883
7	4	A	F	48	2	7.45pm	265	6	1	5.00	67.27	3	132	2.584
8	4	B	F	48	2	7.45pm	265	6	1	5.00	67.27	3		3.037
9	5	A	F	42	2	7.45pm	265	6	3	6.00	44.58	4	154	8.101
10	5	B	F	42	2	7.45pm	265	6	3	6.00	44.58	4		9.240
11	6	A	M	19	2	7.45pm	265	6	4	2.00	58.58	5	150	5.340
12	6	B	M	19	2	7.45pm	265	6	4	2.00	58.58	5	216	8.890
13	7	A	M	16	2	7.45pm	265	6	5	3.00	95.27	6	164	5.863
14	7	B	M	16	2	7.45pm	265	6	5	3.00	95.27	6	200	29.482
15	8	A	M		3	8.00pm	265	6	1	4.00	45.22	1	156	14.035
16	8	B	M		3	8.00pm	265	6	1	4.00	45.22	1	218	10.176
17	10	A	M	34	3	8.00pm	265	6	4	2.00	25.27	7	184	
18	11	A	F	43	3	8.00pm	265	6	6	3.00	42.97	8	200	7.682
19	11	B	F	43	3	8.00pm	265	6	6	3.00	42.97	8	240	30.918
20	12	A	F	48	4	8.15pm	420	5	1	1.00	67.27	3	204	8.926
21	12	B	F	48	4	8.15pm	420	5	1	1.00	67.27	3	176	11.973
22	13	A	M	35	4	8.15pm	420	5	2	4.75	36.37	9	122	4.107
23	13	B	M	35	4	8.15pm	420	5	2	4.75	36.37	9	194	10.638

Appendix 10: Greyhound Phenotypic Data, Heart Rates and Cortisol Measurements.

Sample ID	Dog ID	Sample Type	Sex	Age (months)	Race No.	Time of Race	Race Dist (m)	No. Dogs	Trap No.	Adjusted Rank	Journey Distance (km)	Owner/Trainer	HR (bpm)	Cortisol (ng/ml)
24	14	A	F	24	4	8.15pm	420	5	3	3.50	27.36	10	134	20.877
25	14	B	F	24	4	8.15pm	420	5	3	3.50	27.36	10	120	41.042
26	15	A	F	36	4	8.15pm	420	5	4	2.25	13.52	11	162	13.174
27	15	B	F	36	4	8.15pm	420	5	4	2.25	13.52	11	206	
28	16	A	M	48	4	8.15pm	420	5	5	6.00	1.61	12	144	9.916
29	16	B	M	48	4	8.15pm	420	5	5	6.00	1.61	12		
30	17	A	F	30	5	8.30pm	470	5	2	2.25	5.31	13	140	13.605
31	17	B	F	30	5	8.30pm	470	5	2	2.25	5.31	13	232	16.980
32	18	A	F	36	5	8.30pm	470	5	4	4.75	58.58	5	140	9.070
33	18	B	F	36	5	8.30pm	470	5	4	4.75	58.58	5	178	
34	19	A	M	30	5	8.30pm	470	5	5	3.50	77.41	3	144	6.231
35	19	B	M	30	5	8.30pm	470	5	5	3.50	77.41	3	228	18.166
36	20	A	F	36	5	8.30pm	470	5	6		13.52	11	168	17.552
37	20	B	F	36	5	8.30pm	470	5	6		13.52	11	208	
38	21	A	M	30	6	8.45pm	265	5	1	4.75	90.45	14	210	6.324
39	21	B	M	30	6	8.45pm	265	5	1	4.75	90.45	14	202	27.811
40	22	A	M		7	9.00pm	470	6	1	2.00	30.09	1	178	0.363
41	22	B	M		7	9.00pm	470	6	1	2.00	30.09	1	198	8.688
42	23	A	M		7	9.00pm	470	6	3	5.00		15	234	18.415
43	23	B	M	42	7	9.00pm	470	6	3	5.00		15	194	17.991
44	24	A	F	24	7	9.00pm	470	6	6	1.00	24.14	16	156	12.081
45	24	B	F	24	7	9.00pm	470	6	6	1.00	24.14	16	190	2.101
46	25	A	F	45	8	9.15pm	420	5	1	2.25	25.27	7	172	13.366

Appendix 10: Greyhound Phenotypic Data, Heart Rates and Cortisol Measurements.

Sample ID	Dog ID	Sample Type	Sex	Age (months)	Race No.	Time of Race	Race Dist (m)	No. Dogs	Trap No.	Adjusted Rank	Journey Dist (km)	Owner/Trainer	HR (bpm)	Cortisol (ng/ml)
47	26	A	M	42	8	9.15pm	420	5	2	4.75	3.06	17	188	8.261
48	26	B	M	42	8	9.15pm	420	5	2	4.75	3.06	17	186	12.754
49	27	A	M		9	9.30pm	265	5	2	1.00	5.31	18	188	4.973
50	27	B	M		9	9.30pm	265	5	2	1.00	5.31	18	170	32.104
51	28	A	M	42	9	9.30pm	265	5	3	4.75	60.51	19	150	11.725
52	28	B	M	42	9	9.30pm	265	5	3	4.75	60.51	19	112	15.613
53	29	A	F	36	9	9.30pm	265	5	4	3.50	89.16	20	180	
54	29	B	F	36	9	9.30pm	265	5	4	3.50	89.16	20	210	19.260
55	30	A	M	42	11	10.00pm	265	6	1	1.00	3.06	17	162	9.543
56	30	B	M	42	11	10.00pm	265	6	1	1.00	3.06	17	200	18.249
57	31	A	M	42	11	10.00pm	265	6	2	6.00	81.75	21	178	
58	31	B	M	42	11	10.00pm	265	6	2	6.00	81.75	21	140	39.290
59	32	A	F	30	11	10.00pm	265	6	4	3.00	24.14	16	142	
60	32	B	F	30	11	10.00pm	265	6	4	3.00	24.14	16	132	16.059
61	33	A	F	36	11	10.00pm	265	6	5	4.00	89.16	20	240	3.088
62	33	B	F	36	11	10.00pm	265	6	5	4.00	89.16	20	120	5.589
63	34	A	F	48	12	10.15pm	470	6	1	6.00	40.39	2	200	
64	34	B	F	48	12	10.15pm	470	6	1	6.00	40.39	2	120	8.764
65	35	A	F	47	12	10.15pm	470	6	4	4.00	95.27	6	146	3.686
66	35	B	F	47	12	10.15pm	470	6	4	4.00	95.27	6	192	9.623
67	36	A	M	36	12	10.15pm	470	6	5	3.00	89.16	20	220	7.016
68	36	B	M	36	12	10.15pm	470	6	5	3.00	89.16	20	164	30.640
69	37	A	M	42	12	10.15pm	470	6	6	2.00	58.58	5	198	
70	37	B	M	42	12	10.15pm	470	6	6	2.00	58.58	5	104	23.066

Appendix 10: Greyhound Phenotypic Data, Heart Rates and Cortisol Measurements.

Sample ID	Dog ID	Sample Type	Sex	Age (months)	Race No.	Time of Race	Race Dist (m)	No. Dogs	Trap No.	Adjusted Rank	Journey Dist (km)	Owner/Trainer	HR (bpm)	Cortisol (ng/ml)
71	38	A	F	30	13	10.30pm	470	6	1	1.00	36.53	22	154	6.447
72	38	B	F	30	13	10.30pm	470	6	1	1.00	36.53	22	160	42.781
73	39	A	M	46	13	10.30pm	470	6	2	6.00	47.64	23	198	18.235
74	39	B	M	46	13	10.30pm	470	6	2	6.00	47.64	23	212	32.186
75	40	A	M	30	13	10.30pm	470	6	3	5.00	3.06	17	180	2.328
76	40	B	M	30	13	10.30pm	470	6	3	5.00	3.06	17	128	10.633
77	41	A	M	35	14	7.30pm	265	6	2	2.00	36.37	9	84	9.754
78	41	B	M	35	14	7.30pm	265	6	2	2.00	36.37	9	140	22.956
79	42	A	M	18	14	7.30pm	265	6	6	6.00	35.08	1	120	25.072
80	42	B	M	18	14	7.30pm	265	6	6	6.00	35.08	1	160	30.756
81	43	A	M	42	15	7.45pm	265	6	3	4.00	60.51	19	132	
82	43	B	M	42	15	7.45pm	265	6	3	4.00	60.51	19	124	49.653
83	44	A	M	36	15	7.45pm	265	6	6	6.00	121.67	24	160	50.893
84	44	B	M	36	15	7.45pm	265	6	6	6.00	121.67	24	180	58.861
85	45	A	F	16.5	16	8.00pm	420	5	2	2.25	17.06	25	100	13.444
86	45	B	F	16.5	16	8.00pm	420	5	2	2.25	17.06	25	134	12.318
87	46	A	F	36	16	8.00pm	420	5	4	6.00	13.52	11	148	5.874
88	46	B	F	36	16	8.00pm	420	5	4	6.00	13.52	11	204	22.741
89	47	A	M	20	17	8.15pm	470				75.80	26	92	27.987
90	47	B	M	20	17	8.15pm	470				75.80	26	150	20.838
91	48	A	F	20	17	8.15pm	470	6	5	2.00	108.15	27	170	21.978
92	48	B	F	20	17	8.15pm	470	6	5	2.00	108.15	27		15.797
93	49	A	M	30	17	8.15pm	470	6	6	6.00	67.27	3	150	16.118
94	49	B	M	30	17	8.15pm	470	6	6	6.00	67.27	3	190	11.661

Appendix 10: Greyhound Phenotypic Data, Heart Rates and Cortisol Measurements.

Sample ID	Dog ID	Sample Type	Sex	Age (months)	Race No.	Time of Race	Race Dist (m)	No. Dogs	Trap No.	Adjusted Rank	Journey Dist (km)	Owner/Trainer	HR (bpm)	Cortisol (ng/ml)
95	50	A	F	53	18	8.30pm	420	5	1	2.25	19.96	28	168	7.652
96	50	B	F	53	18	8.30pm	420	5	1	2.25	19.96	28	180	
97	51	A	M	30	18	8.30pm	420	5	5	6.00	60.51	19	124	
98	51	B	M	30	18	8.30pm	420	5	5	6.00	60.51	19	238	13.173
99	52	A	F	54	19	8.45pm	265	6	5	6.00	45.71	29	144	50.755
100	52	B	F	54	19	8.45pm	265	6	5	6.00	45.71	29	136	44.443
101	53	A	F	36	19	8.45pm	265	6	6	1.00	121.67	30	134	17.676
102	53	B	F	36	19	8.45pm	265	6	6	1.00	121.67	30	146	
103	54	A	M	54	20	9.00pm	470	6	2	3.00	36.21	31	128	
104	54	B	M	54	20	9.00pm	470	6	2	3.00	36.21	31	142	92.741
105	55	A	F	36	20	9.00pm	470	6	6	1.00	15.61	17	140	18.032
106	55	B	F	36	20	9.00pm	470	6	6	1.00	15.61	17	208	44.595
107	56	A	M	36	21	9.15pm	265	6	1	2.00	15.61	17	196	9.098
108	56	B	M	36	21	9.15pm	265	6	1	2.00	15.61	17	188	19.162
109	57	A	M	37	21	9.15pm	265	6	2	1.00	75.96	32	144	2.043
110	57	B	M	37	21	9.15pm	265	6	2	1.00	75.96	32	220	40.918
111	58	A	M	24	22	9.30pm	470	5	2	4.75	75.96	5	140	36.869
112	58	B	M	24	22	9.30pm	470	5	2	4.75	75.96	5	174	38.977
113	59	A	M	47	23	9.45pm	265	5	3	1.00	76.93	33	122	11.148
114	59	B	M	47	23	9.45pm	265	5	3	1.00	76.93	33	160	13.700
115	60	A	M	84	24	10.00pm	470	5	4	6.00	36.21	31	120	17.459
116	60	B	M	84	24	10.00pm	470	5	4	6.00	36.21	31	172	20.219
117	61	A	F	30	24	10.00pm	470	5	5	3.50	24.14	16	160	4.335
118	61	B	F	30	24	10.00pm	470	5	5	3.50	24.14	16	188	29.309

Appendix 10: Greyhound Phenotypic Data, Heart Rates and Cortisol Measurements.

Sample ID	Dog ID	Sample Type	Sex	Age (months)	Race No.	Time of Race	Race Dist (m)	No. Dogs	Trap No.	Adjusted Rank	Journey Dist (km)	Owner/Trainer	HR (bpm)	Cortisol (ng/ml)
119	62	A	F	36	25	10.15pm	420	5	1	1.00	4.51	34	106	0.677
120	62	B	F	36	25	10.15pm	420	5	1	1.00	4.51	34	96	20.819
121	63	A	M	36	25	10.15pm	420	5	3	3.50	3.06	17	130	7.930
122	63	B	M	36	25	10.15pm	420	5	3	3.50	3.06	17	192	6.011
123	64	A	F	48	25	10.15pm	420	5	4	4.75	121.67	30	100	11.150
124	64	B	F	48	25	10.15pm	420	5	4	4.75	121.67	30	128	
125	65	A	M	48	25	10.15pm	420	5	5	2.25	121.18	35	154	
126	65	B	M	48	25	10.15pm	420	5	5	2.25	121.18	35	160	7.401
127	66	A	M	27	20	9.00pm	470	6	5	5.00	137.28	36	120	
128	67	B												9.302

Where: *Sample ID*, ID number of the sample; *Dog ID*, ID number of the dog; *Sample Type*, pre-race (A) or post-race (B) sample; *Sex*, male (M) or female (F); *Age*, age of the dog in months; *Race No.*, ID number of the race; *Time of Race*, time the race took place; *Race Dist*, distance of the race in metres (265 m - 470 m) ; *No. Dogs*, number of dogs in the race (4 - 6); *Trap No.*, starting trap number (1 - 6); *Adjusted Rank*, rank finish position adjusted to account for number of dogs in race (1 - 6); *Journey Dist*, distance of the dog's journey to the track on the day of the race, in kilometres; *Owner/Trainer*, ID number of the owner or trainer of the dog (1 - 36); *HR*, Greyhound heart rate in beats per minute; *Cortisol*, Greyhound salivary cortisol concentration in nanograms/millilitre.

Appendix 11: Information sheet for Greyhound owners or trainers considering donation of a saliva sample from a dog for use in the study of salivary cortisol concentrations



Information for Greyhound Owners or Trainers Considering Donation of a Saliva Sample from a Dog

We would like to ask for your help. At the University of Liverpool we carry out research into the health, welfare and performance of Racing Greyhounds. We are carrying out a study to investigate levels of cortisol, a hormone naturally produced by the body in response to stress, in greyhound saliva both before and after racing. This will help us to understand the stress response in greyhounds during racing, why some dogs have a greater stress response than others, and eventually, to find ways to reduce stress and improve greyhound welfare. When we have completed the study, we will communicate everything we learn back to the Greyhound community via owners and trainers forums, Greyhound vets and racing organisations.

To carry out this research, we need to take saliva samples from dogs using special saliva collection swabs. The procedure is very simple and does not cause any harm or distress. We place the swab into the dog's mouth for approximately 90 seconds in order to collect the naturally produced saliva. These samples will give us information about the amount of cortisol hormone present in the saliva at the time of collection, as a measure of stress response in the dog. We also store part of the samples for use in future projects that will benefit greyhound health and welfare. This way we can gain the maximum benefit from the donation.

We would be very grateful if you will agree to donate a saliva sample from your dog. Your involvement is completely voluntary. Your identity, and that of your dog, will remain anonymous and will not be disclosed. Only the people working on this project will have access to individual data.

If you are willing to help us, we need you to sign a simple form (attached) to confirm you have agreed to the donation and you understand what the samples will be used for. We are delighted to talk to you about our work and we really appreciate all of your comments and suggestions. Thank you very much for considering helping us.

Yours sincerely,



Ruth Dockerty BVSc MSc MRCVS

If you have any questions or concerns about this study, please contact Ruth

Dockerty on: *****